The American Journal of Medicine

EDITORIAL BOARD

The American Journal of Medicine

Editor: ALEXANDER B. GUTMAN, M.D.
Professor of Medicine
COLUMBIA UNIVERSITY COLLEGE OF PHYSICIANS AND SURGEONS, NEW YORK
DIRECTOR, DEPARTMENT OF MEDICINE, THE MOUNT SINAI HOSPITAL, NEW YORK

Assistant Editors: MORTIMER E. BADER, M.D. AND RICHARD A. BADER, M.D.
THE MOUNT SINAI HOSPITAL, NEW YORK

ADVISORY BOARD

DAVID P. BARR, M.D.
Professor of Medicine
CORNELL UNIVERSITY MEDICAL COLLEGE
NEW YORK

ARTHUR L. BLOOMFIELD, M.D.
Professor of Medicine, Emeritus
SCHOOL OF MEDICINE, STANFORD UNIVERSITY
SAN FRANCISCO

A. MCGEHEE HARVEY, M.D.
Professor of Medicine
JOHNS HOPKINS UNIVERSITY, SCHOOL OF MEDICINE
BALTIMORE

WALTER L. PALMER, M.D.
Professor of Medicine
UNIVERSITY OF CHICAGO, SCHOOL OF MEDICINE
CHICAGO

ASSOCIATE EDITORS

S. HOWARD ARMSTRONG, JR., M.D., *Chicago*
PAUL B. BEESON, M.D., *New Haven*
J. RUSSELL ELKINTON, M.D., *Philadelphia*
EUGENE B. FERRIS, JR., M.D., *Atlanta*
PETER H. FORSHAM, M.D., *San Francisco*
WILLIAM S. MCCANN, M.D., *Rochester, N. Y.*
GEORGE R. MENEELY, M.D., *Nashville*
CARL V. MOORE, M.D., *St. Louis*
JACK D. MYERS, M.D., *Pittsburgh*
ROBERT E. OLSON, M.D., *Pittsburgh*
DEWITT STETTEN, JR., M.D., *Bethesda*
JOHN V. TAGGART, M.D., *New York*
GEORGE W. THORN, M.D., *Boston*
ROY H. TURNER, M.D., *New Orleans*

The American Journal of Medicine is published monthly by The American Journal of Medicine, Inc., 49 West 45th Street, New York 36, N. Y. Yearly Subscription, $12.00 *U. S. A.;* $13.00 *Canada;* $15.00 *Foreign, including Latin-American countries, Single Numbers* $2.00; *Symposia Numbers* $4.00. *Entered as Second Class Matter June* 28, 1946, *at the Post Office, New York, N. Y., and on June* 28, 1946, *at York, Pa., under the act of March* 3, 1879. *May,* 1957—*Volume* XXII, *No.* 5. *Copyright* © 1957, *by The American Journal of Medicine, Inc.*

MANUSCRIPTS: All manuscripts should be addressed to the Editorial Office of the Journal, 49 West 45th St., New York 36, N. Y. *Style for bibliography:* Doe, J. J. Treatment of hypertension. Am. J. Med., 6: 72, 1948.

Change of address must reach us one month preceding month of issue.

ADVERTISING REPRESENTATIVES

New York: Pliny A. Porter, Parker D. Brewer, Howard S. Schultz —judson 2-3090

Chicago: R. H. Andrew, C. P. Haffner —FRanklin 2-3861
Pasadena: Ren Averill—RYan 1-9291

invitation to asthma?

not necessarily . . .

Tedral, taken at the first sign of attack, often forestalls severe symptoms.

relief in minutes . . . Tedral brings symptomatic relief in a matter of minutes. Breathing becomes easier as Tedral relaxes smooth muscle, reduces tissue edema, provides mild sedation.

for 4 full hours . . . Tedral maintains more normal respiration for a sustained period—not just a momentary pause in the attack.

Tedral provides:

Theophylline	2 gr.
Ephedrine HCl	3/8 gr.
Phenobarbital	1/8 gr.

in boxes of 24, 120 and 1000 tablets

Tedral®

WARNER-CHILCOTT

1 of 3 Survive

Until the advent of Levophed only 1 of 20 pulseless cases of myocardial shock survived. With Levophed 1 of 3 survive.* Use Levophed first in cardiogenic shock.

with **Levophed®** bitartrate

Natural antishock ***pressor*** *hormone*

Every day Levophed reverses "irreversible shock."

*Tainter, M. L.: *Bol. Asoc. méd. Puerto Rico*, 47:305, Aug., 1955.

Winthrop LABORATORIES
New York 18, N. Y.

Levophed (brand of levarterenol), trademark reg. U. S. Pat. Off.

CONTENTS

The American Journal of Medicine

Vol. XXII MAY, 1957 No. 5

Symposium on Inborn Errors of Metabolism

Foreword . . . . . CHARLES E. DENT 671

Mechanisms in Genetic Diseases of Man . . . . . VICTOR A. MCKUSICK 676

Pathogenetic Problems in Phenylketonuria
W. EUGENE KNOX AND DAVID Y.-Y. HSIA 687

Galactosemia . . . . . A. HOLZEL, G. M. KOMROWER AND V. SCHWARZ 703

Sporadic or Non-Endemic Familial Cretinism with Goiter . . . . . .
JOHN B. STANBURY AND EDWARD M. MCGIRR 712

Inborn Errors of Metabolism in Red Cells of Congenital Hemolytic Anemias
T. A. J. PRANKERD 724

Hypophosphatasia . . . . . DONALD FRASER 730

Wilson's Disease. An Inborn Error of Metabolism with Multiple Manifestations
A. G. BEARN 747

The Porphyrias as Inborn Errors of Metabolism . . . . . JAN WALDENSTRÖM 758

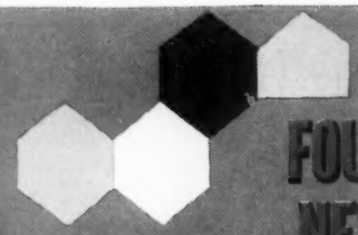
Cystinuria . . . . . H. HARRIS AND E. B. ROBSON 774

Clinical Studies

Hypertrophy of the Right Ventricular Outflow Tract. A Concept of the Electrocardiographic Findings in Atrial Septal Defect
S. GILBERT BLOUNT, JR., E. ADDIS MUNYAN, JR. AND MURRAY S. HOFFMAN 784

The authors demonstrate that the rSR′ pattern with a total QRS time of 0.08 to 0.10 second observed in certain congenital and acquired heart lesions, predominately atrial septal defects, is due to hypertrophy of the right ventricular outflow tract rather than to incomplete right bundle branch block. The evidence for this origin of the rSR′ pattern is adduced by careful studies which include multiple precordial electrocardiograms, serial tracings following cardiac surgery, direct recordings from the surface of the heart and intracavitary electrocardiograms.

Contents continued on page 5



FOUR NEW LONG-ACTING HORMONE PREPARATIONS

DELALUTIN

Squibb Hydroxyprogesterone Caproate

A single injection of this potent new ester provides progestational activity for approximately 2 weeks, when enough estrogen is present. Vials of 2 and 10 cc., each cc. providing 125 mg. hydroxyprogesterone caproate.

DELESTROGEN

Squibb Estradiol Valerate

A single injection provides potent estrogenic action for 2 to 3 weeks, approximating the estrogenic phase of the normal ovarian cycle. Vials of 1 and 5 cc., each cc. providing 10 mg. estradiol valerate.

DELATESTRYL

Squibb Testosterone Enanthate

A single injection provides potent anabolic and androgenic action for 3 to 4 weeks. Vials of 1 and 5 cc., each cc. providing 200 mg. testosterone enanthate.

DELADUMONE

combination of Squibb Testosterone Enanthate and Squibb Estradiol Valerate

A single injection of this precisely balanced dual-hormone formulation provides sustained and integrated anabolic and hormone homeostatic action for 3 to 4 weeks. Vials of 1 and 5 cc., each cc. providing 90 mg. testosterone enanthate and 4 mg. estradiol valerate.

SQUIBB SQUIBB QUALITY—THE PRICELESS INGREDIENT

DELALUTIN®, DELESTROGEN®, DELATESTRYL®, AND DELADUMONE® ARE SQUIBB TRADEMARKS

CONTENTS continued–May 1957

VOLUME TWENTY-TWO NUMBER FIVE

Systemic Manifestations of Hypernephroma. A Review of 273 Cases
LAWRENCE BERGER AND MARVIN W. SINKOFF 791

This review of 273 cases of histologically proved hypernephroma is intended to put the systemic as opposed to the local manifestations of hypernephroma in perspective. A substantial proportion of these patients presented with general systemic and without localizing complaints and some proved to be difficult diagnostic problems, usually but not always solved by urography. The analysis is of special interest in respect to the classic associations with fever, amyloidosis, general debility, primary complaints referable to metastases to the lungs, skeleton, liver and brain.

Seminar on Bone Disease

Osteoporosis . . . . . FREDERIC C. BARTTER 797

Osteoporosis is one of the most commonly encountered disorders of bone and often presents vexing problems in diagnosis, which usually is made by exclusion, and management. The principal defect is in formation of the bone matrix, of which collagen and other proteins are the major constituent; deficiency of bone mineral is a secondary consequence. Dr. Bartter defines and describes the clinical circumstances which are apt to be associated with osteoporosis, including the not inconsiderable group of cases of unknown cause. Treatment, which depends in part upon ascertainable etiology, is outlined—it must often be prolonged, taxing patient and physician alike.

Combined Staff Clinic

Metabolic and Clinical Aspects of Gout 807

Combined Staff Clinic (National Institute of Arthritis and Metabolic Diseases, Bethesda)—This clinic describes some of the more recent work on gout and summarizes current concepts of the pathogenesis of this disorder, and how these concepts have been reflected in improved management. The clinic begins with presentation of an advanced case of tophaceous gout, and discussion of the implications of the manifestations of the disease in such patients. The application of isotope-labeled uric acid and uric acid precursors to study of purine metabolism in normal subjects and those with gout is then described, and the evidence for over-production of uric acid in some subjects with gout is summarized. The final section deals with the uricosuric action of phenylbutazone, probenecid and salicylate.

Case Reports

Isolated ("Primary") Chylopericardium, Due to Anomalous Communications with the Thoracic Duct, of Unknown Causation
WILLIAM M. MADISON, JR. AND BRUCE LOGUE 825

An intriguing case of chylopericardium without chylothorax, a rare condition, is presented. Communication with the thoracic duct was demonstrated by ingenious use of orally ingested lipophilic

Contents continued on page 7

*"...a calmative effect...superior to anything we had previously seen with the new drugs."**

true calmative nostyn®

Ectylurea, AMES
(2-ethyl-*cis*-crotonylurea)

the power of gentleness

allays anxiety and tension
without depression, drowsiness, motor incoordination

NOSTYN is a *calmative*—not a hypnotic-sedative—unrelated to any available chemopsychotherapeutic agent • no evidence of cumulation or habituation • does not increase gastric acidity or motility • unusually wide margin of safety —no significant side effects

dosage: 150-300 mg. (½ to 1 tablet) three or four times daily.

supplied: 300 mg. scored tablets, bottles of 48 and 500.

*Ferguson, J. T., and Linn, F. V. Z.: Antibiotic Med. & Clin. Therapy *3*:329, 1956.

AMES COMPANY, INC · ELKHART, INDIANA
AMES COMPANY OF CANADA, LTD., TORONTO

25057

CONTENTS continued–May 1957

VOLUME TWENTY-TWO NUMBER FIVE

dyes. There was no history of trauma, no etiology could be established. The chylopericardium disappeared after ligation and excision of both major lymphatic channels and all collaterals low in the thorax, together with establishment of drainage through a pericardial window.

Progesterone-Induced Porphyria. Case Report
EDITHE J. LEVIT, JOHN H. NODINE AND WILLIAM H. PERLOFF 831

The authors noted a rather striking association between attacks of acute porphyria, in a patient with acute intermittent porphyria, and endogenously produced or exogenously administered progesterone.

Chronic Disseminated Histiocytosis X (Schüller-Christian Disease) with Pulmonary Involvement and Impairment of Alveolar-Capillary Diffusion
ATTILIO D. RENZETTI, JR., GERARD EASTMAN AND J. HOWLAND AUCHINCLOSS, JR. 834

A well studied case of disseminated histiocytosis with significant pulmonary involvement in addition to bone lesions and diabetes insipidus. Pulmonary function studies reveal the physiologic syndrome of impaired alveolar-capillary diffusion. Improvement on prednisone therapy is described, including the suggestion that much improvement may occur only in patients with predominantly cellular rather than fibrotic reaction.

ERRATUM

In the March, 1957 issue, the Clinico-pathologic Conference, Figures 6 and 7 are interchanged. The photomicrograph set as Figure 7 should appear with the legend for Figure 6 on page 494. The illustration set as Figure 6 should appear with the legend for Figure 7 on page 495.

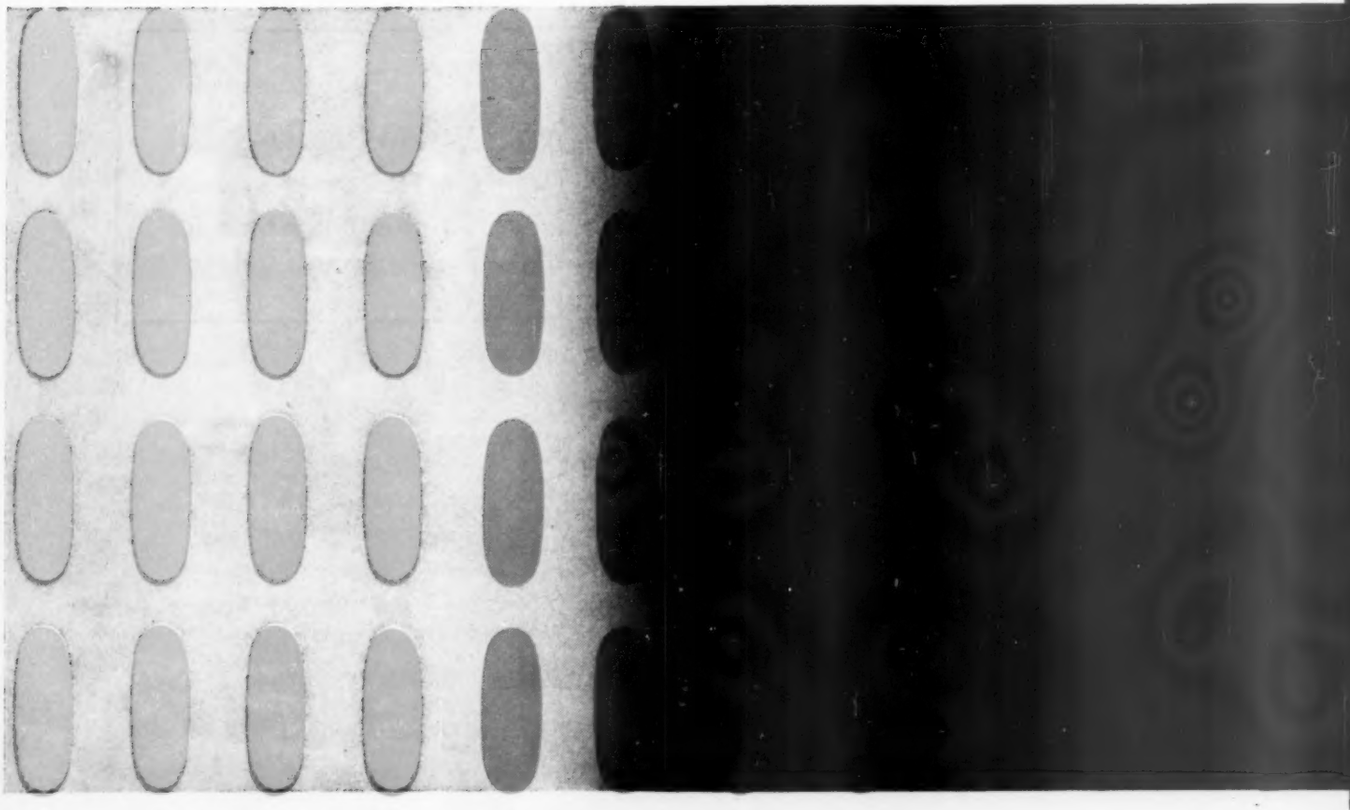
Advertising Index on Page 85

Change of address must reach us one month preceding month of issue.

NEW...

ACHROMYCI

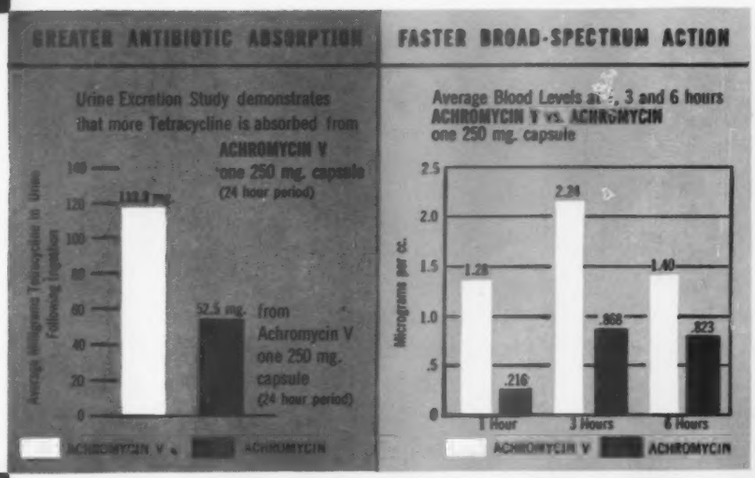
TETRACYCLINE BUFFERED WITH SODIUM



"the only one of its kind"

N*V

METAPHOSPHATE



chemically conditioned for greater clinical efficiency

ACHROMYCIN V admixes sodium metaphosphate with tetracycline. ACHROMYCIN V provides greater antibiotic absorption/faster broad-spectrum action and is indicated for the prompt control of infections, seen in everyday practice, hitherto treated with other broad-spectrum antibiotics.

Available: Bottles of 16 and 100 Capsules.

Each capsule (pink) contains:

Tetracycline equivalent to tetracycline HCl.. 250 mg.

Sodium metaphosphate ..... 380 mg.

ACHROMYCIN V dosage: 6-7 mg. per lb. of body weight per day for children and adults.

LEDERLE LABORATORIES DIVISION
AMERICAN CYANAMID COMPANY
PEARL RIVER, NEW YORK

Lederle

*Reg. U.S. Pat. Off.

designed to

control anxiety

in Arthritis, Asthma, Allergic Dermatoses

with

lower corticoid dosage

the original tranquilizer-corticoid

Ataraxoid*

prednisolone and hydroxyzine

provides the emotional tranquilizer, ATARAX® (hydroxyzine) and the preferred corticoid, STERANE® (prednisolone) • control of emotional factors by tranquilization enhances response to the corticoid for greater clinical improvement • often permits substantial reductions in corticoid dosage, accompanied by reduction of hormonal side effects • confirmed by marked success in 95% of 1095 cases of varied corticoid indications[1]

ATARAXOID *now written as* **Ataraxoid 5.0**

5 mg. prednisolone, 10 mg. hydroxyzine hydrochloride, in green, scored tablets. Bottles of 30 and 100.

and now available as **NEW Ataraxoid 2.5**

2.5 mg. prednisolone, 10 mg. hydroxyzine hydrochloride, in blue, scored tablets. Bottles of 30 and 100.

and **NEW Ataraxoid 1.0**

1.0 mg. prednisolone, 10 mg. hydroxyzine hydrochloride, in orchid, scored tablets. Bottles of 100.

formerly **Ataraxoid**
NOW written **Ataraxoid 5.0**

advantages: (1) greater flexibility of dosage (2) effective tranquilization permits lower corticoid dosage

1. Personal communications

*Trademark

PFIZER LABORATORIES *Division, Chas. Pfizer & Co., Inc. Brooklyn 6, New York*

Pfizer

"Well, then, how about *en brochette?*"

And—while we're stretching a point – what about all those folks who choose one kind of dish, stick with it, eat it all day, every day . . . only to discover (to their chagrin) that they've shortchanged themselves nutritionally? Especially in the important B-complex vitamins. So, for deficiences brought about by unsound mealtime habits or because of illness, senility, stress, or postoperative states, remember SUR-BEX with C. As a dietary supplement: 1 or 2 tablets daily. For postoperative convalescence: 2 or more daily.

Abbott

Just one SUR-BEX tablet a day supplies:

Thiamine Mononitrate	*6 mg.*
Riboflavin	*6 mg.*
Nicotinamide	*30 mg.*
Pyridoxine Hydrochloride	*1 mg.*
Vitamin B_{12} (as cobalamin concentrate)	*2 mcg.*
Calcium Pantothenate	*10 mg.*
Ascorbic Acid	*150 mg.*
Desiccated Liver, N.F.	*300 mg.*
Brewer's Yeast, Dried	*150 mg.*

sur-Bex® with C

ABBOTT'S B-COMPLEX TABLETS WITH C

7CX100

sparine*

HYDROCHLORIDE

10-(γ-dimethylamino-n-propyl)-phenothiazine hydrochloride

*Trademark

Promazine Hydrochloride

THE ALCOHOLIC

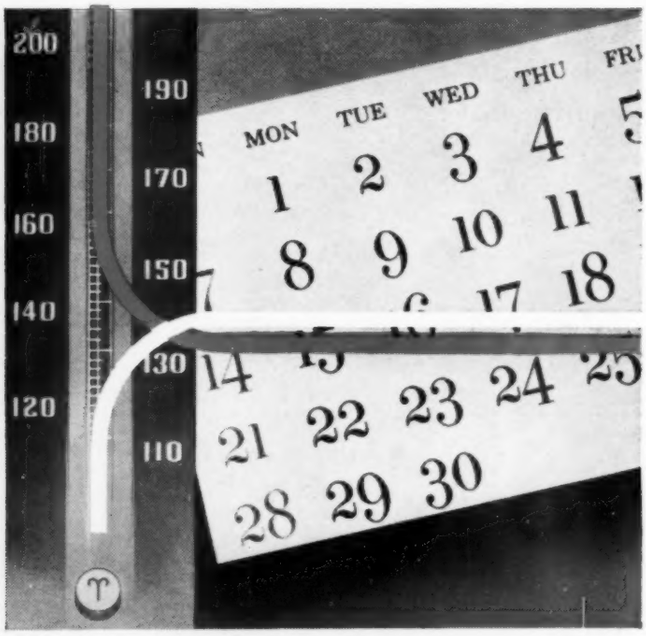
SPARINE is an agent of prompt, predictable, and potent action in controlling withdrawal symptoms.
Often, in selected cases under the adequate supervision of the family physician, it may afford *home control* of postalcoholic agitation and hyperactivity.[1]

SPARINE is well tolerated on intravenous, intramuscular, or oral administration.
Toxicity is minimal—no case of liver damage has been reported. Parenteral use offers (1) minimal injection pain; (2) no tissue necrosis at the injection site; (3) potency of 50 mg. per cc.; (4) no need for reconstitution before injection.

Professional literature available upon request.

1. Figurelli, F.A.: Indust. Med. & Surg. 25:376 (Aug.) 1956.

Wyeth®
Philadelphia 1, Pa.



Predictable hypotensive effect — orally

INVERSINE®

MECAMYLAMINE HYDROCHLORIDE

INVERSINE is chemically different from the quaternary ammonium ganglionic blockers, and orally is completely absorbed. This ensures a predictable, reproducible and lasting hypotensive response—tomorrow's dose of INVERSINE will bring about the same reduction of blood pressure as today's. "This drug is completely absorbed when given by mouth and has such a gradual onset and offset of action that a continuous and effective level of blockade can readily be achieved...."[1]

Reference: 1. J. Michigan Soc. 55:154 (Feb.) 1956.

Dosage: Initial dose, 2.5 mg. twice daily, increased by 2.5 mg. at 2-day intervals. Average daily dose 25-30 mg.
Supplied: 2.5 mg. scored tablets and 10 mg. quarter-sected tablets in bottles of 100.

INVERSINE IS A TRADEMARK OF MERCK & CO., INC.

MSD

MERCK SHARP & DOHME
DIVISION OF MERCK & CO., INC., PHILADELPHIA 1, PA.

Conditions requiring diuretic treatment for sustained periods of time can be ideally controlled by DIAMOX.

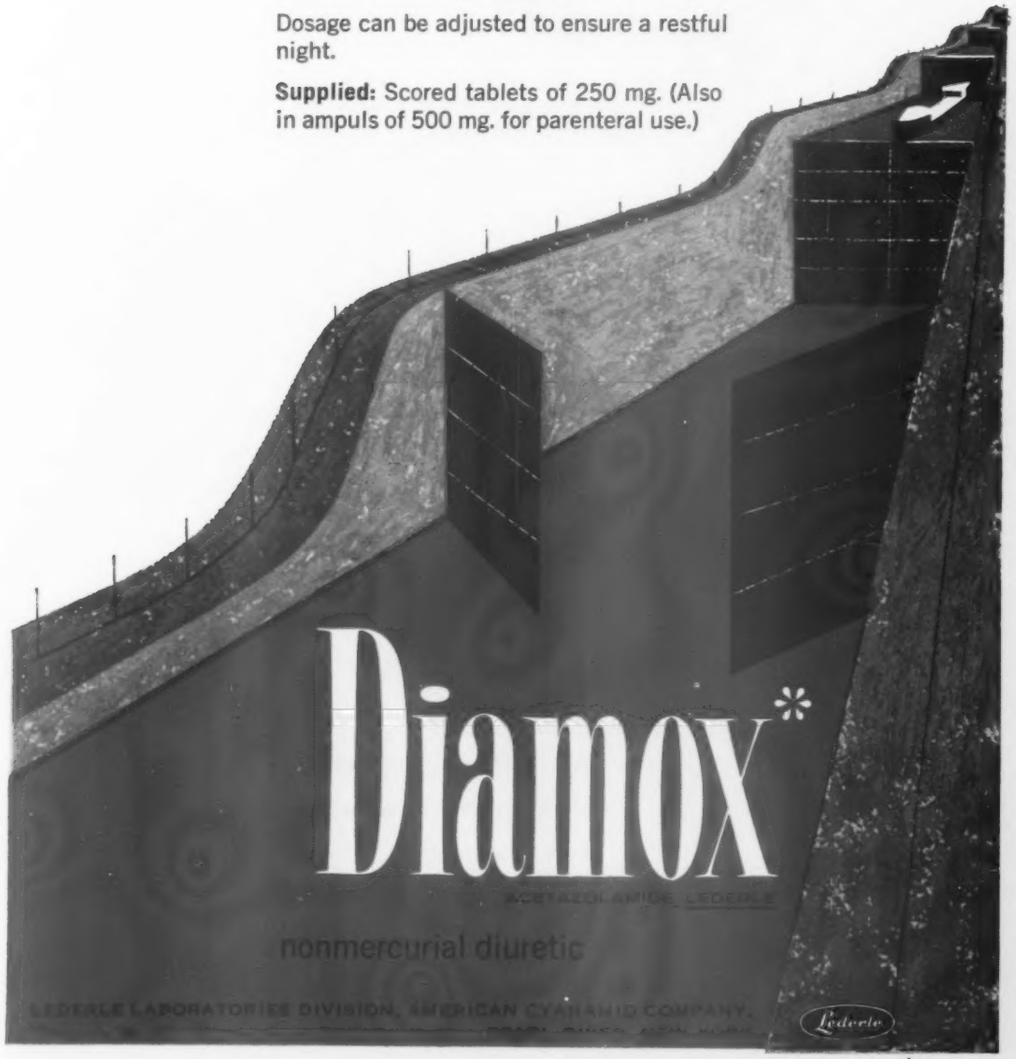
DIAMOX has been found strikingly effective in a variety of conditions: cardiac edema, glaucoma, epilepsy, toxemias of pregnancy, obesity, premenstrual tension.

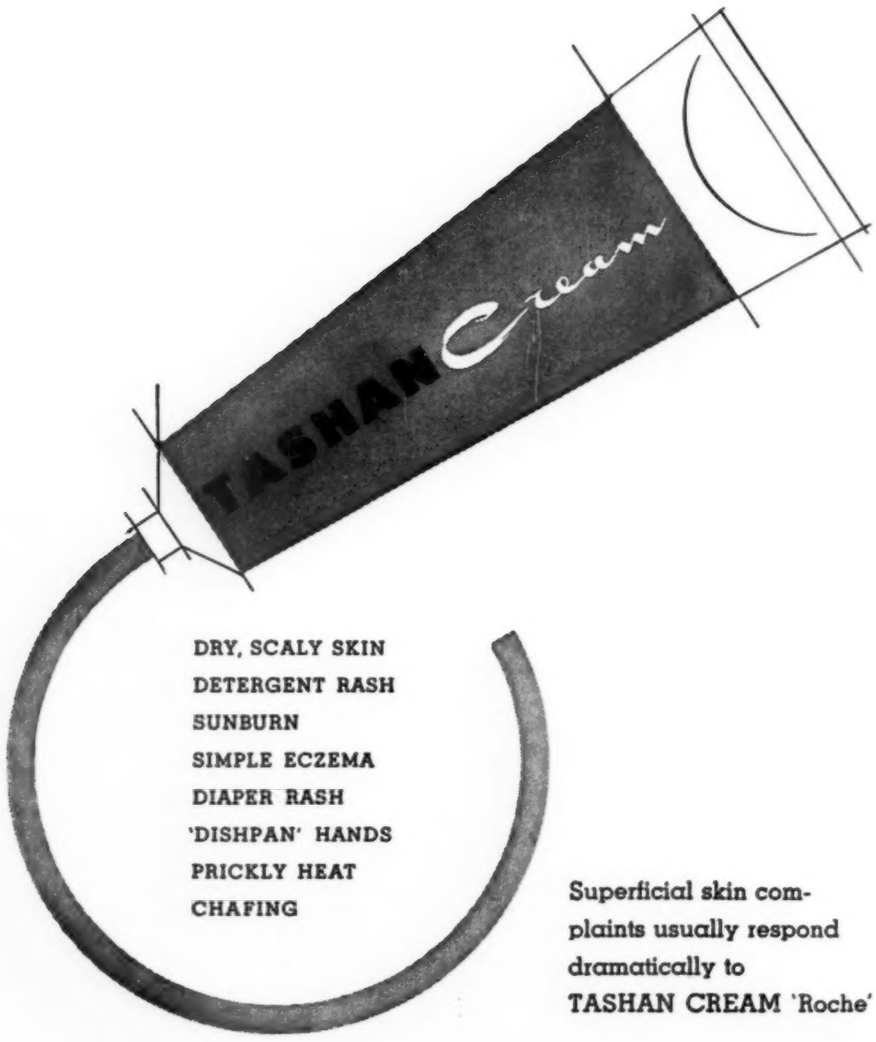
Administration of DIAMOX once daily or every other day results in adequate control of edema since DIAMOX is effective in the **mobilization** of edema fluid and in the **prevention of fluid accumulation.**

A versatile diuretic, DIAMOX is well-tolerated orally, and even when given in long term dosage, side effects are rare. Excretion by the kidney is usually complete within 12 hours with no cumulative effects.

Dosage can be adjusted to ensure a restful night.

Supplied: Scored tablets of 250 mg. (Also in ampuls of 500 mg. for parenteral use.)





Superficial skin complaints usually respond dramatically to TASHAN CREAM 'Roche'

Antiprurient, soothing, and healing—contains vitamins A, D, E, and *d*-Panthenol, in a cosmetically pleasing water-soluble base which fastidious patients will enjoy using. Hoffmann-La Roche Inc., Nutley, N. J.

TASHAN T. M.

CIBA

Serpasil

The growing use of Serpasil®
(reserpine CIBA)
in everyday practice

One of the safest, least toxic and most effective therapeutic agents for many conditions in which the weaker tranquilizers or sedatives are inadequate.

On the following pages you will find information on these aspects of Serpasil therapy:

PAGE	
2	emotional disorders
3	hypertension
4	tachycardia
4	acute hypertensive crises
5	alcoholism
5	pediatric emotional problems
5	acute psychotic disturbances
6	side effects and precautions

in emotional disorders

Serpasil® provides true emotional control

In your daily practice there are undoubtedly many patients whose degree and type of emotional disturbance—characterized by overexcitation, anxiety and agitation—can *not* be adequately controlled with sedatives or weaker tranquilizers. These are the patients whom you can help most with once-a-day administration of Serpasil. For Serpasil actually sets up a "stress barrier" against anxiety and tension the patient would otherwise find intolerable. With Serpasil you can control the emotional turmoil of disturbed individuals; and because Serpasil is restricted to prescription use, control remains in your hands.

Although it is a first choice in hypertension, Serpasil does not significantly lower blood pressure in normotensive patients.

USUAL DOSE: Initial range is 0.1 mg. to 0.5 mg. (two 0.25-mg. tablets) daily. As little as 0.1 mg. is sufficient for maintenance in some patients. Serpasil can be given in a single daily dose.

"...relieves anxiety and irritability and calms the patient so effectively that because of this latter property alone, the drug should remain in the medicinal armamentarium."

Finnerty, F. A., Jr., and Sites, J. G.: Am. J. M. Sc. 229:379 (April) 1955.

in hypertension

Serpasil® can always be considered first

BECAUSE *alone:* Serpasil successfully reduces blood pressure, slowly and safely, in about 70 per cent of cases of mild to moderate hypertension.[1]

BECAUSE *as a "primer":* Serpasil may be advantageously used to begin antihypertensive therapy, however severe the case, since it gently adjusts the patient to the physiologic setting of lower pressure.

BECAUSE *as a "background" agent throughout other therapy:* Serpasil permits lower dosage of the more potent antihypertensives needed for refractory cases, thus minimizing the incidence and severity of side effects.

USUAL DOSE: Initially, two 0.25-mg. tablets. After a week, daily dose should be reduced to 0.25 mg. or less for maintenance.

"...a useful agent for the treatment of certain types of hypertension....The action...was increased by combining it with [Apresoline]..."[2]

1. Coan, J. P., McAlpine, J. C., and Boone, J. A.: J. South Carolina M. A. 51:417 (Dec.) 1955.
2. Winsor, T.: Ann. New York Acad. Sc. 59:61 (April 30) 1954.

in tachycardia

Serpasil® slows the rapid heart

Many patients can benefit from the heart-slowing action of Serpasil. Those in whom tachycardia is deleterious are helped by its unique bradycardic effect, for Serpasil prolongs diastole and allows more time for the myocardium to rest. Blood flow and cardiac efficiency are thus enhanced.

USUAL DOSE: 0.1 mg. to 0.5 mg. (two 0.25-mg. tablets) daily. After one or two weeks dose may be reduced.

"Reserpine [Serpasil] was found useful in relieving the tachycardia and emotional symptoms associated with cardiac arrhythmias, thyrotoxicosis, neurocirculatory asthenia, and even coronary heart disease."

Halprin, H.: J. M. Soc. New Jersey 52:616 (Dec.) 1955.

in acute hypertensive crises

Parenteral Serpasil

Serpasil can be used alone in hypertensive emergencies or as a background to more potent antihypertensive agents. Its antihypertensive action is prompt and well-tolerated.

USUAL DOSE: 2.5 mg. (1 ml.) intramuscularly. Additional intramuscular doses of 2.5 mg. may be given as necessary every 8 to 24 hours.

"...appears to be [a] treatment of choice for hypertensive crises."

Griffin, R. W., Stover, J. W., and Ford, R. V.: New England J. Med. 254:593 (March 29) 1956.

in alcoholism

Serpasil® relieves drink-inducing tension

As a part of long-term therapy, oral Serpasil helps the alcoholic "stay on the wagon" by relieving drink-inducing tension, making him more amenable to your counseling.

In acute alcoholism, delirium tremens can generally be controlled within 24 hours with parenteral Serpasil...without the addicting or soporific dangers of drugs such as paraldehyde.

USUAL DOSE: *Chronic phase:* two 0.25-mg. tablets or less daily. *Acute phase:* two 2.5-mg. parenteral doses (1 ml. each) 3 or more hours apart. Occasionally, repeat injections of 2.5 mg. every 4 to 6 hours may be necessary.

> *"...the tranquilizing and anxiety-relieving properties of this drug [Serpasil] offer the possibilities of its being extremely helpful for the long-term therapy of the chronic alcoholic."*

Greenfield, A. R.: Am. Pract. & Digest Treat. 7:241 (Feb.) 1956.

in pediatric emotional problems

Serpasil Elixir benefits the "problem child"

Serpasil provides a shield against stress in the overreactive, tense, "problem child." Striking remissions have been observed in children with excessive crying, poor eating and sleeping patterns.

USUAL DOSE: 0.1 to 0.3 mg. daily (½ to 1½ teaspoons of Serpasil Elixir, 0.2 mg. per 4-ml. teaspoon).

> *"...provided dramatic relief in remitting the syndrome of irritability in 29 of the 32 cases studied..."*

Talbot, M. W., Jr.: Ann. New York Acad. Sc. 61:188 (April 15) 1955.

in acute psychotic disturbances

Parenteral Serpasil

The family physician is often called to subdue and arrange for quick hospitalization of patients who suddenly experience violent psychotic episodes. With intramuscular Serpasil these patients are quickly tranquilized and rendered amenable to 'quiet' hospitalization.

USUAL DOSE: 5 mg. intramuscularly followed, if necessary, by another 5-mg. intramuscular dose in 90 minutes.

> *"It is now possible to discreetly manage acutely disturbed psychiatric patients by the prompt administration of adequate doses of reserpine (Serpasil)."*

Ayd, F. J., Jr.: The Pharmacologic Management of Everyday Psychiatric Problems (A Scientific Exhibit). Presented at the Clinical Meeting of the American Medical Association, Boston, Mass., Nov. 29-Dec. 2, 1955.

Serpasil®: side effects and precautions

The side effects of Serpasil are characteristic of all rauwolfia preparations.

Although millions of patients have taken Serpasil over the past several years, very few serious side reactions have been reported. There have been no cases of blood dyscrasia, liver damage, addiction or withdrawal symptoms. When patients are properly selected and the lowest effective maintenance dose is established, the physician can prescribe Serpasil confidently, with little fear of untoward reactions.

Depression

Mental depression, which has developed in a small percentage of patients treated with rauwolfia, should be differentiated from the transient change in mood or physical fatigue that is experienced by almost everyone in the general population. It should also be distinguished from the lethargy experienced by some patients on rauwolfia therapy.

In the few cases in which mental depression does occur, there is some question as to whether or not it is a direct effect of rauwolfia. According to Mayo Clinic investigators,[1] the evidence indicates that rauwolfia *per se* does not cause depression, but rather that it unmasks an underlying susceptibility to depressive reactions. Kinross-Wright[2] states: "It is likely that depression will occur only in a predisposed individual or in one who is already mildly depressed." Ayd,[3] in a (very) recent paper, states: "That this drug may cause depression is uncertain. After reviewing a large number of so-called drug-induced depressions it appears that in some cases what was called depression was excessive tranquilization, while in the rest, the patients were depressed before the drug was started, and what the drug did was make the depression more apparent."

Whether or not it is an effect of rauwolfia, physicians and responsible members of the patient's family should be on the alert for the development of symptoms of depression, particularly in patients with a history of pre-existing depressive tendencies. Daily doses above 0.25 mg. are contraindicated in the latter group. On withdrawal of rauwolfia, depression usually disappears, but active treatment, including hospitalization for shock therapy, has been required in some cases.

Adjunctive use of mood-elevating agents such as Ritalin is often sufficient to reverse mild depressions or drug-induced lethargy.

Other side effects

In addition to lassitude or drowsiness, other mild side effects of Serpasil include occasional nasal stuffiness and increased frequency of defecation and/or looseness of stools. Rarely, anorexia, headache, bizarre dreams, nausea and dizziness occur. With parenteral Serpasil there is a possibility of marked hypotensive effect; therefore, the blood pressure should be taken before injection and the patient kept under observation for 5 or 6 hours thereafter. Because initial doses above 0.3 mg. tend to increase gastric secretion of hydrochloric acid, daily doses above 0.25 mg. are contraindicated in patients with a history of peptic ulcer and lower doses should be used with caution.

For further details on side effects and precautions, write Medical Service Division.

1. Litin, E. M., Faucett, F. L., and Achor, R. W. P.: Proc. Staff Meet., Mayo Clin. 31:233 (April 18) 1956.
2. Kinross-Wright, V.: Wisconsin M. J. 55:1073 (Oct.) 1956.
3. Ayd, F. J., Jr.: Presented at the Sesquicentennial Convention of The Medical Society of The State of New York, New York City, Feb. 18, 1957.

SUPPLIED:

TABLETS, 0.1 mg., 0.25 mg., 1 mg., 2 mg. and 4 mg.

ELIXIRS, 0.2 mg. and 1 mg. per 4-ml. teaspoon.

PARENTERAL SOLUTION: *Ampuls*, 2 ml., 2.5 mg. Serpasil per ml. *Multiple-dose Vials*, 10 ml., 2.5 mg. Serpasil per ml.

APRESOLINE® hydrochloride (hydralazine hydrochloride CIBA)
RITALIN® hydrochloride (methylphenidate hydrochloride CIBA)

CIBA
SUMMIT, N. J.

2/2443MK

. . . the eighteen pound, transistorized model 300 VISETTE

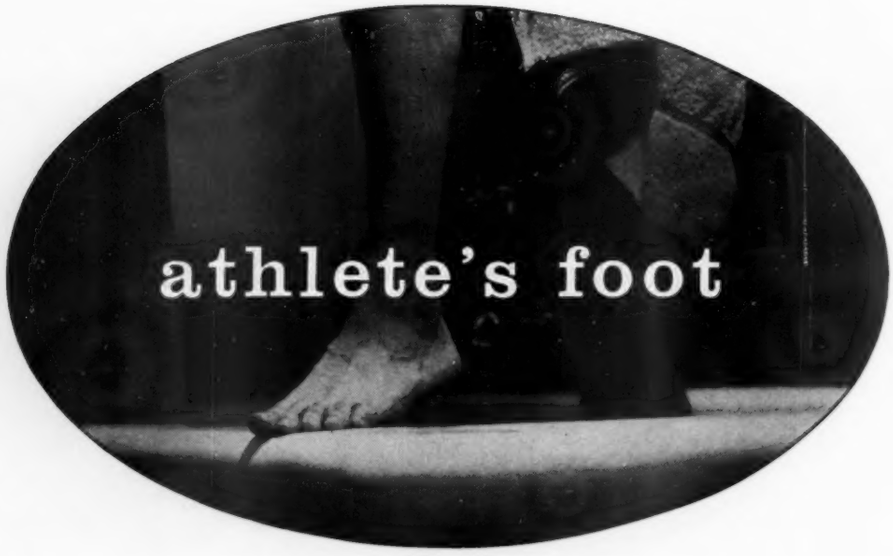
For the clinical accuracy your heart practice demands . . . *and a degree of portability never before approached in the field of 'cardiography* . . . this new Sanborn instrument offers a truly remarkable answer.

In the VISETTE you will find outstanding Sanborn quality and performance, achieved through the latest electronic techniques and the most modern principles of instrumentation. Tiny transistors largely replace bulky vacuum tubes . . . entire circuits are contained in plug-in printed wiring panels no larger than a playing card . . . 'cardiograms are clearly traced on chart paper in a new, convenient width. Innovations such as these have also made possible economies in production, reflected in the comparably lower price of the new 300 VISETTE.

Every design feature, every component in this modern instrument, serves a single purpose: clinically *accurate* 'cardiograms with the greatest possible *convenience*. The "Sanborn man" in or near your city can provide complete details, and a demonstration in your office if you wish. And of course you may try a VISETTE (as you can other Sanborn instruments)—*before* buying, without cost or obligation.

To those who already own the famous Model 51 Viso-Cardiette, the new VISETTE can be an invaluable "companion" ECG—especially suited to use outside the office, or in hospital wards. Or, for those who prefer a larger instrument, using conventional 6 cm. width recording paper, the "51" is still available at $785 delivered.

SANBORN COMPANY
WALTHAM 54, MASS.



carrier unto himself

Once he is infected with athlete's foot, he is likely to remain a "carrier unto himself," even without re-exposure. Daily routine application of Desenex protects against reinfection and recurrence.

fast relief from itching
prompt antimycotic action
continuing prophylaxis

Desenex®
OINTMENT and SOLUTION
Buffered at pH 6.5
POWDER

Maltbie

For most effective and convenient therapy and continuing prophylaxis, use Desenex as follows:

AT NIGHT the Ointment (zincundecate) — 1 oz. tubes and 1 lb. jars.

DURING THE DAY the Powder (zincundecate) — 1½ oz. and 1 lb. containers.

AFTER EVERY FOOT BATH the Solution (undecylenic acid)—2 fl. oz. and 1 pt. bottles. Use only when skin is unbroken.

In otomycosis, Desenex solution or ointment.

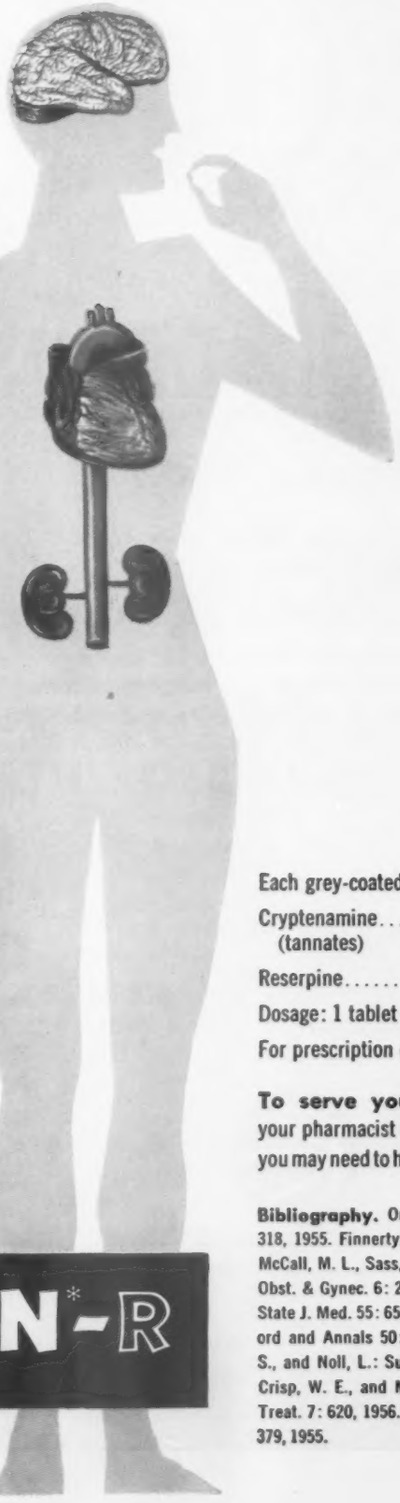
Write for free sample supply to Professional Service Department.

MALTBIE LABORATORIES DIVISION • WALLACE & TIERNAN, INC. • Belleville 9, N. J.

PD-71

HYPERTENSION

"ACTING IN CONCERT"†



to treat

the hypertensive patient

as a whole

Unitensen-R combines cryptenamine and reserpine which "act in concert" to control the entire syndrome of essential hypertension.

Cryptenamine dependably lowers blood pressure, and improves cerebral and renal circulation. It also increases cardiac efficiency, and may arrest the progress of vascular damage.

Reserpine raises the threshold of emotional response and stifles neurogenic aggravation of the disease.

Given together, cryptenamine and reserpine produce a far better therapeutic effect than when given separately. And successful therapy is usually maintained with dosages well below those producing side effects.

†Cohen, B. M.; Cross, E. B., and Johnson W.: Am. Prac. & Digest Treat. 6: 1030, 1955.

Each grey-coated Unitensen-R tablet contains:

Cryptenamine (tannates) ..... 1.0 mg.

Reserpine ..... 0.1 mg.

Dosage: 1 tablet t.i.d.

For prescription economy, prescribe in 50's.

To serve your patients today—call your pharmacist for any additional information you may need to help you prescribe Unitensen-R.

Bibliography. Orgain, E. S.: Postgrad. Med. 17: 318, 1955. Finnerty, F. A.: Am. J. Med. 17: 629, 1954. McCall, M. L., Sass, D. K., Wagstaff, C., and Cutler, J.: Obst. & Gynec. 6: 297, 1955. Cohen, B. M.: New York State J. Med. 55: 653, 1955. LaBarbera, J. F.: Med. Record and Annals 50: 242, 1956. Voskian, J.; Assali, N. S., and Noll, L.: Surg., Gynec. & Obst. 102: 37, 1956. Crisp, W. E., and McCall, M. L.: Am. Prac. & Digest Treat. 7: 620, 1956. Finnerty F. A.: Am. J. M. Sc. 229: 379, 1955.

UNITENSEN*-R

*T.M., Reg. U.S. Pat. Off.

IRWIN, NEISLER & COMPANY

DECATUR, ILLINOIS

THE PICTURE OF COMFORT — ALL THROUGH THE PREGNANCY
if she's blue at breakfast...
BONADOXIN®
(brand of Meclizine HCl and Pyridoxine HCl)
stops morning sickness
Controlled studies indicate that BONADOXIN relieves symptoms-quickly-in 9 of every 10 gravida. Tolerance is excellent.
Prescribe: One tablet at bedtime. Severe cases, one tablet at bedtime, one on arising. In tiny pink-and-blue tablets, bottles of 25 and 100. ℞ only.
if she needs
a nutritional buildup—and
freedom from leg cramps†
prescribe STORCAVITE®
Phosphate-free calcium, iron, 10 essential vitamins, 8 important minerals.
Usually 3 tablets daily, with meals. In bottles of 100.
†when due to a high phosphorous intake..
ROERIG
℞
CHICAGO 11, ILLINOIS
PEACE of mind ATARAX®

when you prescribe

CARBRITAL®

you prescribe sleep

PARKE, DAVIS & COMPANY • DETROIT 32, MICHIGAN

50108

Beginning July 1957 **THE AMERICAN JOURNAL OF MEDICINE** *Presents*

SEMINAR ON ATHEROSCLEROSIS

1. *July*—Cholesterol and Phospholipid Metabolism
   DR. DONALD ZILVERSMIT
   Univ. of Tennessee
   Dept. of Physiology
   Memphis, Tenn.
2. *August*—Serum Lipoproteins and Their Relation to Atherosclerosis
   DR. HOWARD A. EDER
   Albert Einstein Medical Center
   Bronx, N. Y.
3. *September*—Epidemiology of Atherosclerosis
   DR. GEORGE MANN
   200 Oakland Street
   Wellesley Hill, Mass.
4. *October*—Genetic Aspects of Atherosclerosis
   DR. EDWIN O. WHEELER
   Mass. General Hospital
   Boston, Mass.
5. *November*—Hormonal Influences on Serum Lipids
   DR. DAVID ADLERSBERG
   Mount Sinai Hospital
   New York, N. Y.
6. *December*—Effects of Various Dietary Fats on Serum Lipids
   DR. EDWARD H. AHRENS
   Rockefeller Institute
   New York, N. Y.

THE AMERICAN JOURNAL OF MEDICINE
49 West 45th Street **New York 36, New York**

can you read this thermometer,

doctor?

Naturally not. Missing calibration makes it worthless.

Equally useless and dangerous is a "quantitative" urine-sugar test that does not quantitate dependably, or omits readings in the critical range.

Enzyme urine-sugar tests are sensitive and specific for glucose—excellent "yes" or "no" tests but undependable for quantitation. King and Hainline,[1] after testing 1,000 urines, found an enzymatic urine-sugar test unable to distinguish in the important range between ½ per cent and 2 per cent or more of urinary glucose. Leonards,[2] in a report on 4,020 tests, revealed that "...in 502 out of 804 tests the wrong interpretation was made." He concluded that enzymatic urine-sugar testing "...as a quantitative procedure is unsatisfactory and can lead to serious error in the interpretation of a patient's clinical condition."[2]

Failure to recognize this limitation of enzyme tests may result in incorrect insulin dosage,[2] and may lead to diabetic complications.

(1) King, J. W., and Hainline, A., Jr.: Commercial Glucose Oxidase Preparations for the Detection of Glucose in Urine, Cleveland Clin. Quart. **23**:212, 1956. (2) Leonards, J. R.: Evaluation of Enzyme Tests for Urinary Glucose, J.A.M.A. **163**:260 (Jan. 26) 1957.

reliable readings throughout the critical range—
does not omit ¾% (++) and 1% (+++)

color
calibrated
CLINITEST®
BRAND

a 15 year "standard" in urine-sugar testing

AMES COMPANY, INC • ELKHART, INDIANA • Ames Company of Canada, Ltd., Toronto

38157

relaxes both mind and muscle

for anxiety and tension in everyday practice

- well suited for prolonged therapy
- well tolerated, relatively nontoxic
- no blood dyscrasias, liver toxicity, Parkinson-like syndrome or nasal stuffiness
- chemically unrelated to phenothiazine compounds and rauwolfia derivatives
- orally effective within 30 minutes for a period of 6 hours

For treatment of **anxiety and tension states and muscle spasm**

THE ORIGINAL MEPROBAMATE

Miltown®

2-methyl-2-n-propyl-1,3-propanediol dicarbamate—U. S. Patent 2,724,720

Tranquilizer with muscle-relaxant action

NOW IN 2 CONVENIENT DOSAGES

NEW 200 mg. SUGAR-COATED TABLETS

STANDARD 400 mg. SCORED TABLETS

DISCOVERED AND INTRODUCED BY WALLACE LABORATORIES, *New Brunswick, N. J.*

SUPPLIED : *(Bottles 50 tablets)*
400 mg. scored tablets
200 mg. sugar-coated tablets

USUAL DOSAGE : *One or two 400 mg. tablets t.i.d.*

Literature and Samples Available on Request

THE MILTOWN® MEPROBAMATE MOLECULE

CM-3707-R5

to minimize
morning joint stiffness...

PERSISTIN*

Night-long salicylate therapy with a single dose of Persistin at bed-time helps prevent "joint jelling" in arthritic patients.

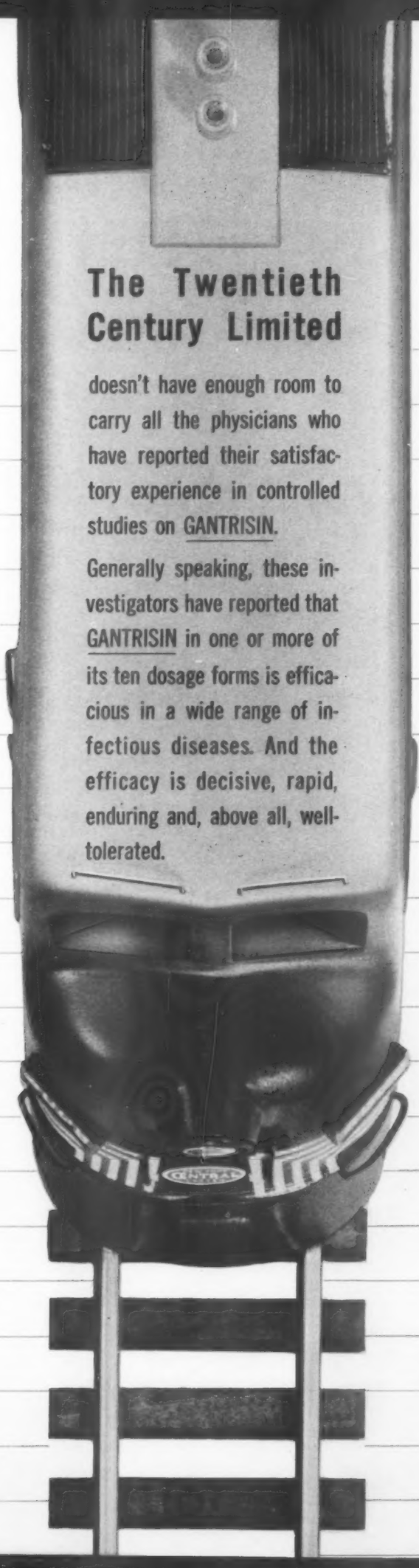
Each Persistin tablet contains acetylsalicylic acid 2½ gr. (160 mg.) and salicylsalicylic acid 7½ gr. (480 mg.). The latter ingredient is slowly absorbed and eliminated for prolonged salicylate action up to 8 hours.

Complete dosage information in PDR . . . bottles of 90 tablets

Samples and literature on request

Sherman Laboratories
Detroit 11, Michigan

*Trademark—Pat. Pend.



The Twentieth Century Limited

doesn't have enough room to carry all the physicians who have reported their satisfactory experience in controlled studies on GANTRISIN.

Generally speaking, these investigators have reported that GANTRISIN in one or more of its ten dosage forms is efficacious in a wide range of infectious diseases. And the efficacy is decisive, rapid, enduring and, above all, well-tolerated.

Gantrisin
with a plus

Azo Gantrisin

in urinary tract infections

AZO GANTRISIN 'ROCHE'

prompt, specific action against pain

and infection . . . in one tablet

ACTION:
Azo Gantrisin is an antibacterial-analgesic agent specifically designed for treatment of urinary tract infections. The high plasma and urine levels of Gantrisin act systemically and locally to clear both descending and ascending infections. Painful burning, urgency and nocturia are relieved – often within 2 hours – by the analgesic action of phenylazo-diamino-pyridine HCl upon the mucosa of the lower urinary tract.

USES:
For antibacterial therapy and local pain relief in urinary tract infections; prevention of infection after cystoscopy, catheterization and other instrumentation. Also useful after urologic surgery.

ADVANTAGES:
Wide antibacterial spectrum . . . high plasma levels . . . high urine levels . . . high solubility (even in acid urine) . . . no need for alkalies . . . no likelihood of renal blocking . . . local pain relief.

DOSAGE:
Adults (and children over 100 lbs) – 2 tablets, q.i.d.
Children under 100 lbs – 1 tablet, q.i.d.

Caution: The usual precautions in sulfonamide therapy should be observed. Because Azo Gantrisin contains phenylazo-diamino-pyridine HCl, it is contraindicated in glomerular nephritis, pyelonephritis of pregnancy with gastrointestinal symptoms, severe hepatitis and uremia. In such cases, Gantrisin should be used alone.

SUPPLIED:
Red, monogrammed tablets, each containing 0.5 Gm Gantrisin plus 50 mg phenylazo-diamino-pyridine HCl; bottles of 100 and 500 tablets.

Gantrisin®– brand of sulfisoxazole

ROCHE

HOFFMANN - LA ROCHE INC • NUTLEY • N. J.

WHEN HER SWEET TOOTH TROUBLES HER DIGESTION

TAKA-COMBEX®

to help her cope with carbohydrates
avoid vitamin deficiencies

TAKA-COMBEX Kapseals®—containing the starch-digestant Taka-Diastase,® B vitamins, ascorbic acid, and liver concentrates—are available in bottles of 100 and 1,000.

TAKA-COMBEX Elixir—containing Taka-Diastase and B vitamins—is available in 16-ounce bottles.

PARKE, DAVIS & COMPANY · DETROIT 32, MICHIGAN

80114

The American Journal of Surgery
publishes the papers of

Three Surgical Societies

The Pacific Coast Surgical Association
The American Society of Maxillofacial Surgeons
The American Association for the Surgery of Trauma

The American Journal of Surgery, Inc.
49 West 45th Street, New York 36, New York

FAST RELIEF *is essential*

WIGRAINE®

RELIEVES MIGRAINE QUICKLY

If taken at the first indication of prodromal symptoms, Wigraine relieves migraine headaches in a matter of minutes. And because the Wigraine tablet disintegrates quickly, and acts promptly, less medication is required to control the complete syndrome.

Wigraine combines, in an uncoated tablet, ergotamine tartrate and caffeine to control vascular headache; belladonna alkaloids for nausea and vomiting; and acetophenetidin to relieve occipital muscle pain.

Formula: Each Wigraine tablet contains 1 mg. ergotamine tartrate, 100 mg. caffeine, 0.1 mg. of belladonna alkaloids (levorotatory)*, and 130 mg. acetophenetidin.

Supplied: Individually foil-stripped and packaged in boxes of 20. Send for complete descriptive literature.

**87.5% hyoscyamine, 12.5% atropine, as sulfate.*

Organon INC.
ORANGE, N. J.

your patients with generalized gastrointestinal complaints need the comprehensive benefits of

Tridal®

(DACTIL® + PIPTAL®—in one tablet)

rapid, prolonged relief throughout the G.I. tract
with unusual freedom from antispasmodic
and anticholinergic side effects

LAKESIDE

One tablet two or three times a day and one at bedtime. Each TRIDAL tablet contains 50 mg. of Dactil, the *only* brand of N-ethyl-3-piperidyl diphenylacetate hydrochloride, and 5 mg. of Piptal, the *only* brand of N-ethyl-3-piperidyl-benzilate methobromide.

14387

Your restless patients' sleep problems
can be managed conservatively

prescribe **NOCTEC**

Squibb Chloral Hydrate

"The general practitioner likes it..."
"...can be given to patients of all ages and physical status..."

"...patients with cardiac disease..."
"...no proof that it is deleterious to the heart..."

"The psychiatrist often finds it the agent of choice..."
"...much less likely to produce mental excitement..."

"...frequently the favorite of the dermatologist..."
"...skin reactions from it are uncommon..."

Current Concepts in Therapy: Sedative-Hypnotic Drugs.
II. Chloral Hydrate. New England J. Med. 255:706 (Oct. 11) 1956.
adults: 1 or 2 7½ gr. capsules or 1 or 2 teaspoonfuls of Noctec Solution 15 to 30 minutes before bedtime.
children: 1 or 2 3¾ gr. capsules or ¼ to 1 teaspoonful of Noctec Solution 15 to 30 minutes before bedtime.
7½ and 3¾ gr. capsules, bottles of 100.
Solution, 7½ gr. per 5 cc. tsp., bottles of 1 pint.

SQUIBB

Squibb Quality—the Priceless Ingredient "NOCTEC"® IS A SQUIBB TRADEMARK

Doctor, do you feel that your colleagues are missing important new medical findings by not reading The American Journal of Medicine?

Let us send them a complimentary copy—of course there is no obligation to you or your colleagues. Just fill out the coupon and mail it to us.

*Dr.*_____ *Your Name*_____

*Address*_____ *Address*_____

_____ _____

The American Journal of Medicine

49 West 45th Street · New York 36, New York

prepare your "over-forty" patient for his future...

ELDEC* Kapseals

mineral-vitamin-hormone supplement

to aid in maintaining nutritional and hormonal efficiency

Available in bottles of

PARKE, DAVIS & COMPANY · DETROIT 32, MICHIGAN

Three essential steps in establishing correct eating patterns:

1 SUPERVISION BY THE PHYSICIAN[1,2,3]

2 A BALANCED EATING PLAN[1,2,3]

In the development and maintenance of good eating habits, there are three essentials: support and supervision by the physician, a balanced eating plan, and selective medication.[1,2,3]

3 SELECTIVE MEDICATION[1,2,3]

OBEDRIN PROVIDES:

- Methamphetamine for its anorexigenic and mood-lifting effects.
- Pentobarbital as a balancing agent, to guard against excitation.
- Vitamins B_1 and B_2 plus niacin to supplement the diet.
- Ascorbic acid to aid in the mobilization of tissue fluids.

Since Obedrin contains no artificial bulk, the hazards of impaction are avoided. The 60-10-70 Basic Plan provides for a balanced food intake, with sufficient protein and roughage.

1. Eisfelder, H.W.: Am. Pract. & Dig. Treat. *5*:778 (Oct. 1954).
2. Freed, S.C.: G.P. *7*:63 (1953).
3. Sherman, R.J.: Medical Times, *82*:107 (Feb. 1954).

Obedrin®

and the 60-10-70 Basic Plan

FORMULA:

Semoxydrine HCl (Methamphetamine HCl) 5 mg.; Pentobarbital 20 mg.; Ascorbic acid 100 mg.; Thiamine mononitrate 0.5 mg.; Riboflavin 1 mg.; Niacin 5 mg.

Write for 60-10-70 Menu pads, weight charts and clinical supply of Obedrin.

THE S. E. MASSENGILL COMPANY

BRISTOL, TENNESSEE

NEW YORK • KANSAS CITY • SAN FRANCISCO

'Roche' announces...

Gantrimycin

GANTRISIN PLUS OLEANDOMYCIN

Here is antibacterial cross fire to check many systemic and local infections. Gantrimycin combines the modern, broad-spectrum sulfonamide, Gantrisin, with the new and dramatic antibiotic, oleandomycin.

Gantrimycin is effective against both gram-positive and gram-negative organisms. Of special significance . . . its antibacterial spectrum includes staphylococci which display increasing resistance to penicillin and most other antibiotics . . . a timely and well calculated approach to the mounting problem of drug resistant pathogens. Gantrimycin is well tolerated with little evidence of cross resistance with most other antibiotics.

Each Gantrimycin tablet contains 333 mg Gantrisin and 75 mg oleandomycin (in the form of the phosphate salt); supplied in bottles of 50.

ROCHE HOFFMANN - LA ROCHE INC • NUTLEY • N. J.

Gantrisin® —brand of sulfisoxazole Gantrimycin T.M.

Ecolid

FOR SEVERE—INCLUDING MALIGNANT—HYPERTENSION

ECOLID is a powerful, orally effective ganglionic blocking agent. In some patients it has dramatically reversed the course of severe hypertension and prolonged their lives. ECOLID has been shown to produce a longer lasting, smoother and more consistent and predictable response than either pentolinium or hexamethonium. However, as with all ganglionic blocking agents, the patient must be carefully managed. Before instituting treatment with ECOLID, it is advised that the physician be thoroughly familiar with this drug's effects as well as side effects. Complete literature may be obtained from the Medical Service Division, CIBA, Summit, New Jersey.

SUPPLIED:
Tablets (Rotocotes), 25 mg. (ivory) and 50 mg. (pink).
New Form of Issue: 10 mg. (orange).

ECOLID® chloride (chlorisondamine chloride CIBA)
ROTOCOTES™ (compressed, dry-coated tablets CIBA)

CIBA
SUMMIT, N.J.

keep

the "reducer"

happy

'Methedrine'®

brand Methamphetamine Hydrochloride

'Methedrine' dispels abnormal craving for food, and subtly elevates the mood. Reducing diets are accepted easily, without frustration.

'Methedrine' is safe, in recommended doses, for pregnant women.

'Methedrine' brand Methamphetamine Hydrochloride Tablet of 5 mg., scored.

BURROUGHS WELLCOME & CO. (U.S.A.) INC., Tuckahoe, New York

Announcing a unique new rauwolfia derivative...

First report on one of the most encouraging advances in psychopharmacology since the introduction of rauwolfia: a tranquilizing-antihypertensive agent which combines the potency of the rauwolfias with significantly fewer and milder side effects.

In mid-1955, Abbott Laboratories released for clinical trial a new alkaloid of *Rauwolfia canescens*. This new alkaloid, later named Harmonyl, received special attention because of the high potency and low toxicity it exhibited in pharmacological testing.

Since that time, Harmonyl has been tried in conditions ranging from mild anxiety to major mental illnesses and in hypertension. Every charactertistic of the drug was studied . . . evaluated . . . compared. And from the reports, one fact stands out:

- **In more than two years of clinical trials, Harmonyl has exhibited significantly fewer and milder side effects in comparative studies with reserpine. This, while demonstrating effectiveness comparable to the most potent forms of rauwolfia.**
- **Most significant: Harmonyl causes less mental and physical depression. *And there are very few reports of the lethargy seen with many other rauwolfia preparations.***

This is not to suggest, of course, that side effects will not occur with Harmonyl—as with any potent therapeutic agent. But the mildness of side effects, in the few instances in which they have been reported, suggests Harmonyl as a drug of choice in conditions ranging from mild anxiety to major mental illness and in hypertension.

Why fewer and less severe side effects?

Some investigators suggest that the evidence of less parasympathetic effect with Harmonyl in animals might also be true in man. In chronic toxicity studies with Harmonyl this was manifested by less diarrhea, "bloody tears" and ptosis in rats than was observed with the same dosage level of reserpine. Dogs also exhibited milder side effects—in particular, diarrhea. No organ toxicity or hematological change occurred over a wide dosage range.

Harmonyl as a tranquilizer

While Harmonyl's safety is most impressive, clinical investigators have reported other notable characteristics for this wide-range tranquilizer. For instance, following an eight-month study of chronic, hospitalized mental patients, Ferguson[1] reported:

Harmonyl*

(Deserpidine, Abbott)

• Harmonyl benefited at least 15% more overactive patients than oral reserpine.

• Harmonyl was more potent in controlling aggression, requiring only one-half to two-thirds the dosage of reserpine to achieve equal therapeutic effect.

• A number of patients experiencing side reactions during treatment with reserpine were completely relieved when changed to Harmonyl.

In his summary, Ferguson concluded: *"The most notable impressions were the absence of side effects and relatively rapid onset of action with Harmonyl."*

Harmonyl in hypertension

Hypertension studies show that the average reduction in blood pressure obtained with Harmonyl compares closely to that obtained with reserpine. The tranquilizing effect of the two drugs also appeared similar, except that few cases of giddiness, vertigo, sense of detached existence or disturbed sleep were seen with Harmonyl.

Dosages In mild anxiety, as little as 0.1 mg. of Harmonyl a day may be effective. In institutionalized psychiatric patients, not less than 2 to 3 mg. a day is likely to be beneficial.

In mild essential hypertension, treatment may be started with one 0.25 mg. Harmonyl tablet three or four times a day. After about ten days (or sooner, depending upon response), dosage may be reduced. A maintenance dose of 0.25 mg. daily is often sufficient.

Precautions, Contraindications As with other forms of rauwolfia, Harmonyl must be used cautiously in peptic ulcer and epilepsy and in patients about to undergo surgery or electroshock treatment. Despite the infrequency of reports involving depression, patients with a history of depressive episodes should be watched carefully.

Professional literature is available upon request.

Supplied: Harmonyl is supplied in 0.1-mg., 0.25-mg. and 1-mg. tablets.

Abbott

Reference: 1. Ferguson, J. T. Comparison of Reserpine and Harmonyl in Psychiatric Patients: A Preliminary Report, Journal Lancet, 76:389, December, 1956.

*Trademark

705145

Synatan®

much less CNS overstimulation . . .

less than any other anorexic you're using . . .

only 3.7% of 699 patients on proper dosage schedule exhibited CNS overstimulation with Synatan *(tanphetamin)**

Synatan

tanphetamin protocolloid complex, Irwin, Niesler

the smoothest amphetamine compounds you can use . . .

Seco-Synatan™

Synatan with secobarbital

each Synatan tabule contains tanphetamin (dextro-amphetamine tannate) 17.5 mg., equivalent to 5.25 mg. of *d*-amphetamine base.

Seco-Synatan contains Synatan 17.5 mg. and secobarbital 35.0 mg.

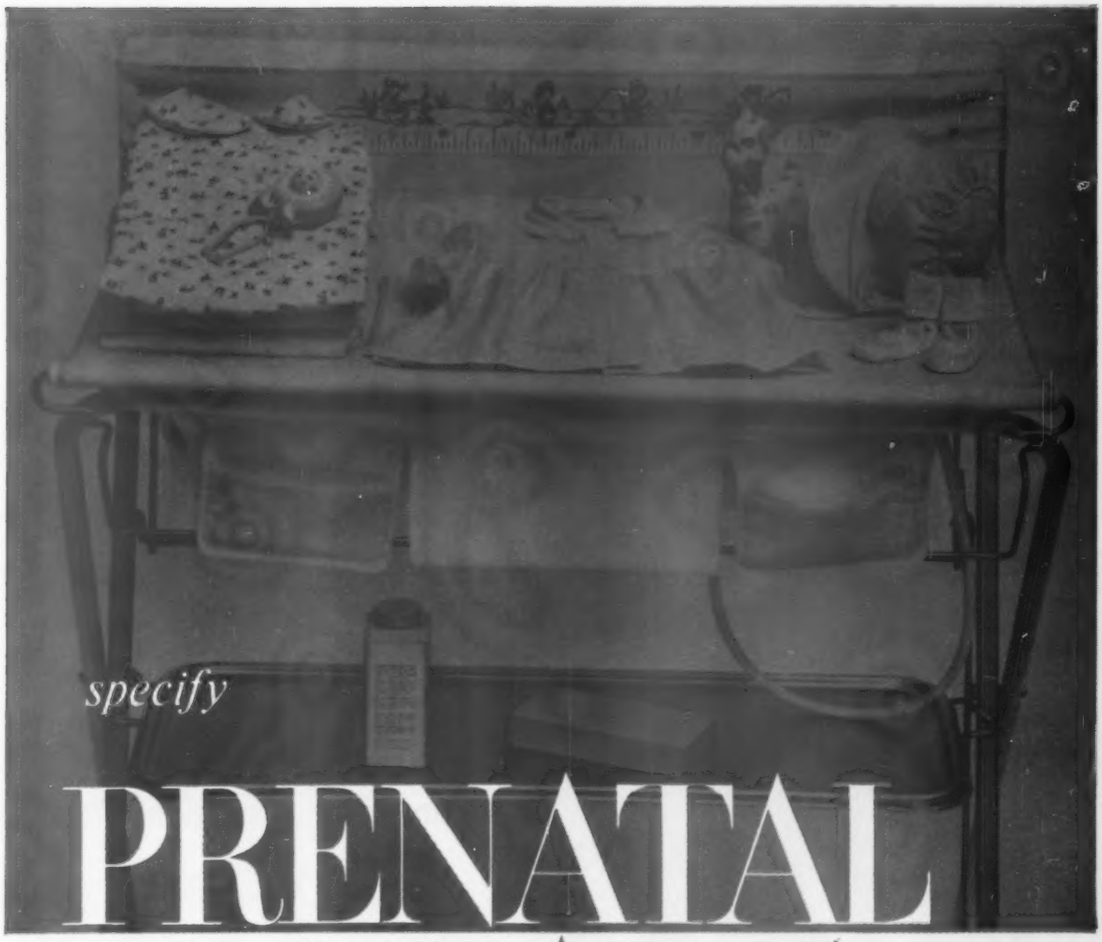
dosage: One or two tabules at 10:00 a.m. for all day control.

To serve your patients today—
call your pharmacist for any additional information you may need to help you prescribe Seco-Synatan and Synatan.

For prescription economy, prescribe Synatan and Seco-Synatan in 50's.

*Garrett, T. A.: Clin. Med. 3: 1185 (Dec.) 1956.

IRWIN, NEISLER & CO. • DECATUR, ILLINOIS



specify

PRENATAL DRI-KAPS*

VITAMINS—MINERALS

dry filled
sealed capsules

to meet additional dietary requirements throughout pregnancy and lactation

Patients prefer PRENATAL DRI-KAPS because they are dry-filled, easy-to-swallow and leave no aftertaste.

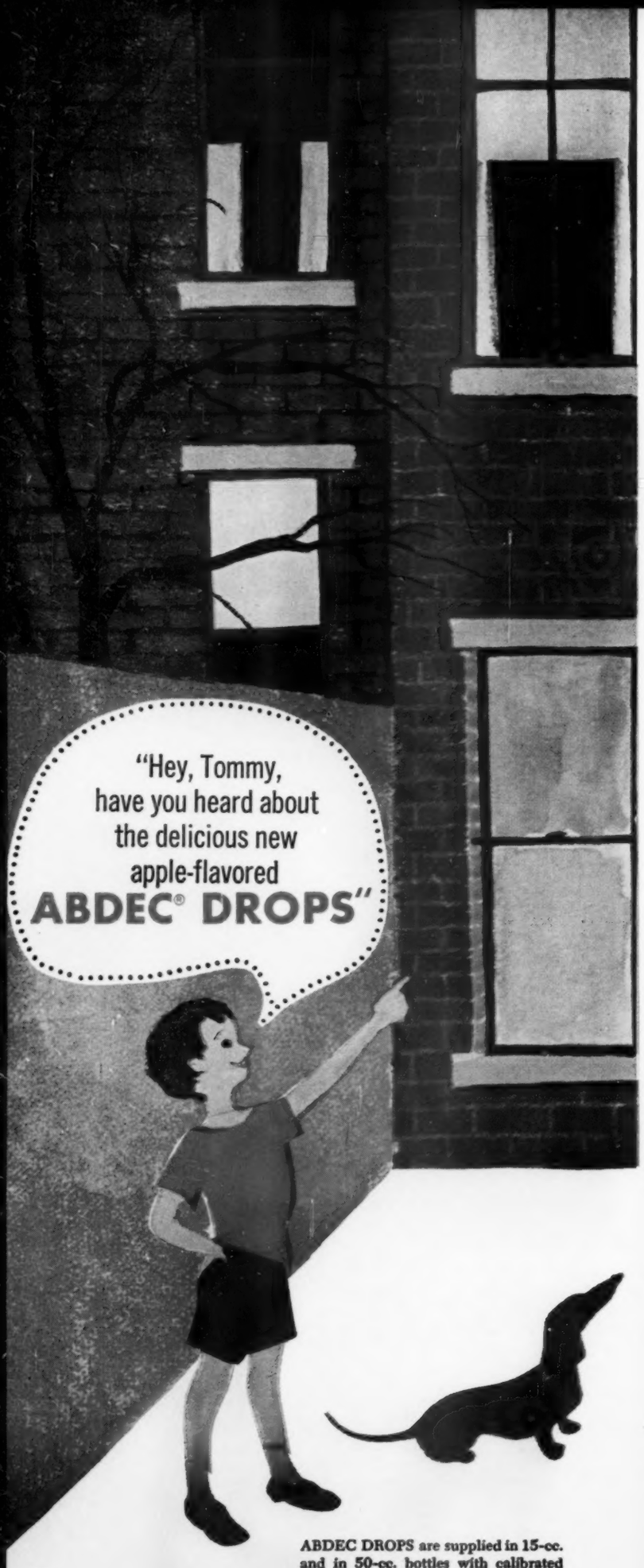
Physicians prescribe PRENATAL DRI-KAPS because they can be certain of: Comprehensive Multivitamin—Multimineral Supplementation • Specific Antianemia Factors • More Rapid and Complete Absorption • Convenient Dosage (1 to 3 Capsules Daily)

Each capsule contains:

Vitamin A	2000 U.S.P. Units
Vitamin D	400 U.S.P. Units
Thiamine Mononitrate (B_1)	2 mg.
Riboflavin (B_2)	2 mg.
Niacinamide	7 mg.
Vitamin B_{12}	1 mcgm.
Vitamin K (Menadione)	0.5 mg.
Ascorbic Acid (C)	35 mg.
Folic Acid	1 mg.
Calcium (in $CaHPO_4$)	250 mg.
Phosphorus (in $CaHPO_4$)	190 mg.
Dicalcium Phosphate Anhydrous ($CaHPO_4$)	869 mg.
Iron (in $FeSO_4$)	6 mg.
Ferrous Sulfate Exsiccated	20 mg.
Manganese (in $MnSO_4$)	0.12 mg.

Lederle

LEDERLE LABORATORIES DIVISION
AMERICAN CYANAMID COMPANY
PEARL RIVER, NEW YORK *TRADEMARK—DRY-FILLED CAPSULES



ABDEC DROPS are supplied in 15-cc. and in 50-cc. bottles with calibrated non-breakable plastic droppers.

Coming Soon . . .

The

American Journal of Medicine

TEN YEAR INDEX

July 1946 through June 1956

This subject and author index provides an invaluable aid for quick reference and review purposes to the articles and authors who have appeared in the journal during the past ten years.

ORDER FORM

The American Journal of Medicine, Inc.
49 West 45th Street, New York 36, N. Y.

Please send me the new Ten Year Index to The American Journal of Medicine for which I enclose $3.00 U.S.A.—$3.50 Foreign

Name .....

Address .....

City .....Zone .....State .....

(New York City residents, add 3% sales tax)

REPRINT ORDER FORM

THE AMERICAN JOURNAL OF MEDICINE
49 W. 45th St., New York 36, N. Y.

Please send me the following Seminars reprinted from the AMERICAN JOURNAL OF MEDICINE:

☐ GASTROINTESTINAL PHYSIOLOGY	$2.00
☐ BLOOD COAGULATION	$2.00
☐ ANTI HYPERTENSIVE DRUGS	$2.00
☐ HEMOLYTIC ANEMIAS	$2.00
☐ CARBOHYDRATE METABOLISM	$2.00
☐ ALLERGY	$2.00
☐ DISEASES OF THE PANCREAS	$2.00

Enclosed is my check

Name_____

Address_____

City_____ State_____

*translation: "It so happens that highly palatable, *apple-flavored* ABDEC DROPS are, at present, my source of nutritional supplementation, providing ample amounts of eight important vitamins in aqueous, nonalcoholic form."

PARKE, DAVIS & COMPANY
Detroit 32, Michigan

01857

NEOBON®

TO FIGHT THE INROADS OF AGE

Current opinion stresses the importance of early recognition of the undesirable effects of aging, and adequate metabolic support of the body's fight against them.[1] NEOBON, by providing 4 factors PLUS 1, corrects all 5 of the recognized treatable causes of aging.

Gonadal Hormone Decline—NEOBON'S daily dose of 3 mg. Methyltestosterone and 0.018 mg. Ethinyl Estradiol offsets it.

Hematinic Deficiencies—NEOBON combats nutritional anemia and iron deficiency with essential hematinic factors.

Digestive Enzyme Deficiency—NEOBON supplies pepsin and pancreatin to insure proper absorption and utilization of foods—despite digestive "let-down" of aging.

Nutritional Inadequacy—NEOBON'S complete combination of essential minerals and vitamins replaces deficiencies inherent in the restricted diets of the aging.

PLUS—NEOBON'S new lysine, the amino acid that lifts low value vegetable proteins to the high grade quality found in meat and eggs.

NEOBON in bottles of 60 soft, soluble capsules; prescription only.

1. Klemme, H. L.: Clin. Med., October, 1956.

NEW NEOBON® LIQUID, *a geriatric tonic providing gonadal and thyroid supplementation, improved carbohydrate and protein utilization, hematinic action, and mild antidepressant effect.*

In 16 oz. bottles; prescription only.

PEACE of mind ATARAX®

ROERIG Chicago 11, Illinois

Relief

comes quickly

for itching, burning,

scaling scalps . . .

once you prescribe Selsun®

Why is Selsun the most effective treatment known for seborrheic dermatitis of the scalp?

Selsun relieves itching and burning with the first few applications.

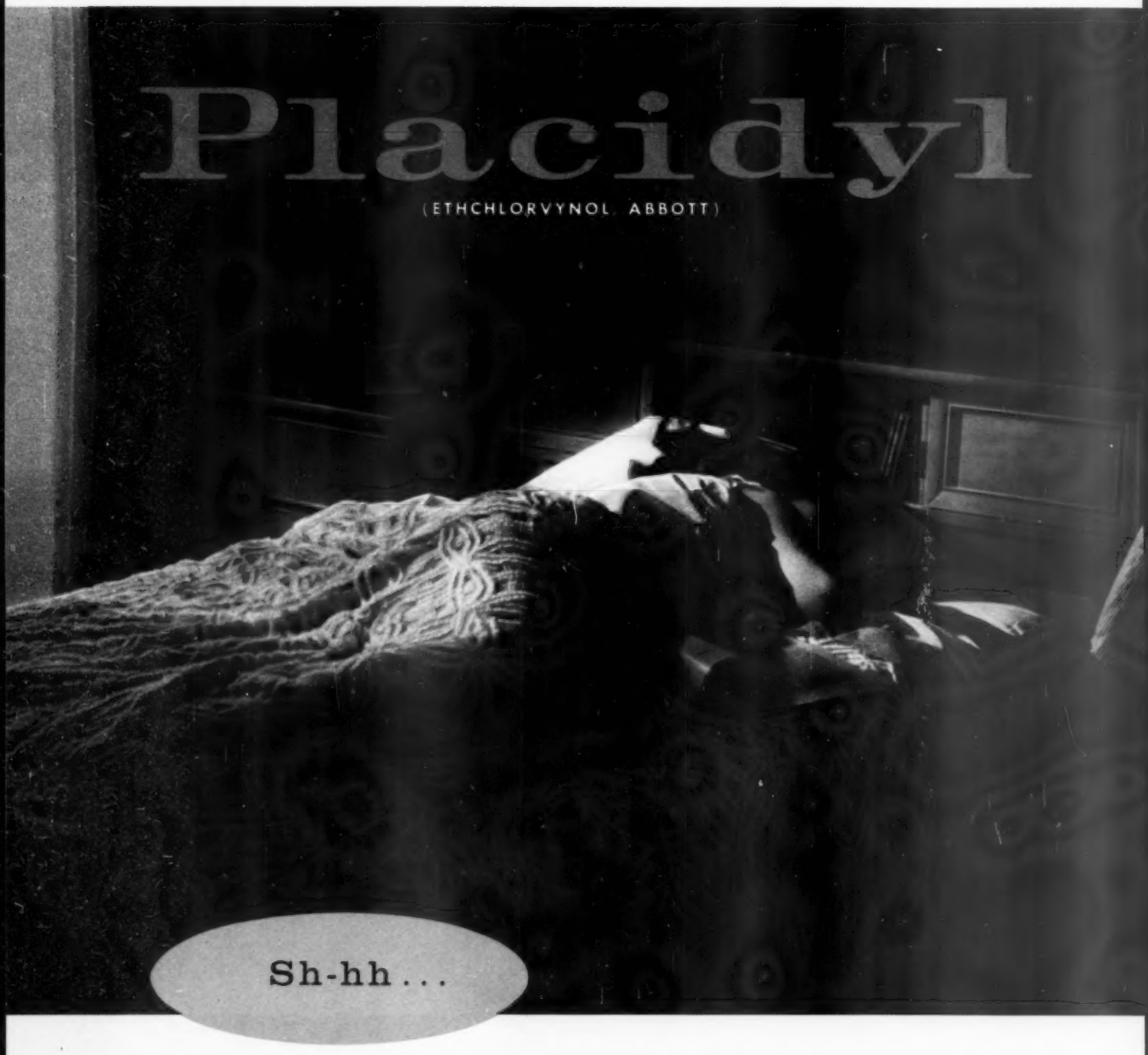
Then, Selsun completely controls scaling—in 81-87% of seborrheic dermatitis, 92-95% of dandruff cases.

And relief lasts up to four weeks between applications, with few remissions when Selsun therapy is continued as needed. Selsun is sold in 4-fluidounce plastic bottles with directions, on prescription only.

Abbott

®Selsun—Selenium Sulfide, Abbott

705005



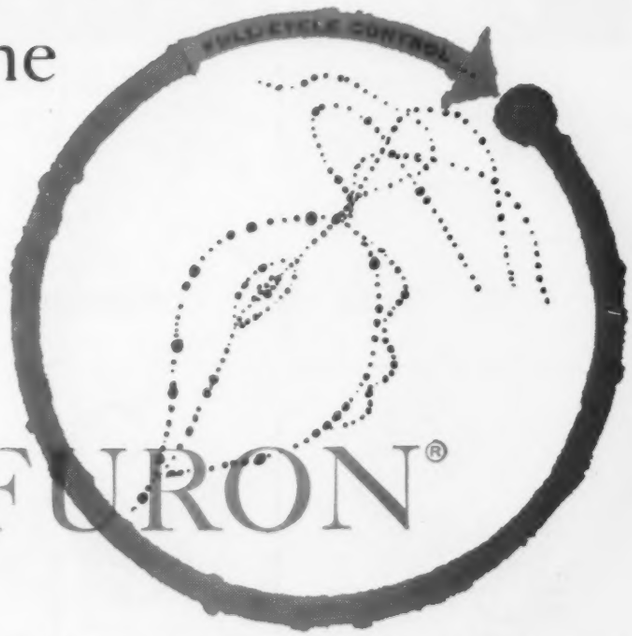
NUDGES YOUR PATIENT TO SLEEP

Use Placidyl to relieve simple insomnia without need of barbiturates. You'll find it especially desirable for helping patients sleep during periods of worry, mild excitement, domestic or business strain, and the like. Excellent, too, for daytime tranquilizing and muscle relaxation.

100-mg., 200-mg., and 500-mg. capsules, bottles of 100.

Abbott

Even stubborn trichomoniasis yields... because Tricofuron is effective during menstruation, the critical time for therapy.



TRICOFURON®

Recurrences of trichomoniasis "are most likely to follow the menstrual period."[1]

"Over and over again today patients are seen with what is said to be an intractable, treatment-resistant Trichomonas infestation, but history-taking often reveals that such patients have never had treatment prescribed during any menstrual period."[2]

Menstrual blood in the vagina "forms an excellent medium for the rapid multiplication of T. vaginalis"[3] and "lowers the acidity of the vagina and hence there is a tendency to recrudescence [of trichomoniasis] at that time."[4]

Tricofuron is powerfully trichomonacidal "even in the presence of vaginal debris and menstrual blood."[3]

For 44 of 48 patients: lasting cure was obtained with a single course of Tricofuron therapy.[3]

Vaginal Suppositories—for home use—each morning and night through one cycle, including the important menstrual days. Contain 0.25% Furoxone® (brand of furazolidone) in a water-miscible base. Box of 12, each sealed in green foil.

Vaginal Powder—for office use—applied by the physician at least once a week, except during menstruation. Contains 0.1% Furoxone in an acidic powder base of lactose, dextrose, citric acid and a silicate. Bottle of 30 Gm.

References: 1. Bernstine, J. B., and Rakoff, A. E.: Vaginal Infections, Infestations and Discharges, New York, The Blakiston Company, Inc., 1953, p. 235. 2. Overstreet, E. W.: Arizona M. **10**:383, 1953. 3. Schwartz, J.: Obst. Gyn., N. Y. **7**:312, 1956. 4. Crossen, R. J.: Diseases of Women, St. Louis, The C. V. Mosby Company, 1953, p. 292.

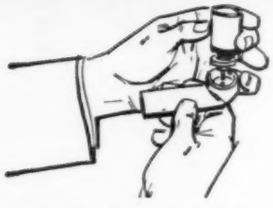
EATON LABORATORIES *NORWICH, NEW YORK*

Nitrofurans—a new class of antimicrobials—neither antibiotics nor sulfonamides

MILLIONS OF ASTHMATIC ATTACKS

have been aborted faster...more effectively... more economically with

Medihaler®



SIMPLE TO USE

CONVENIENT

SUITABLE FOR CHILDREN, TOO

SLIPS INTO POCKET OR PURSE

Automatically measured dosage and true nebulization...nothing to pour or measure...One inhalation usually gives prompt relief of acute or recurring asthmatic attacks.

Medihaler-Epi replaces injected epinephrine in urticaria, edema of glottis, etc. due to acute food, drug or pollen reactions...Each 10 cc. bottle delivers 200 inhalations.

IN ASTHMA PRESCRIBE EITHER

Medihaler-EPI® Riker brand epinephrine U.S.P. 0.5% solution in inert, nontoxic aerosol vehicle. Each measured dose 0.12 mg. epinephrine. In 10 cc. bottle with measured-dose valve.

Medihaler-ISO® Riker brand isoproterenol HCl 0.25% solution in inert, nontoxic aerosol vehicle. Each measured dose 0.06 mg. isoproterenol. In 10 cc. bottle with measured-dose valve.

Note: First prescription for Medihaler medications should include the desired medication and Medihaler Oral Adapter (supplied with pocket-sized plastic carrying case for medication and Adapter).

The Medihaler Principle

is also available in Medihaler-Nitro™ (octyl nitrite) for the rapid relief of angina pectoris ...and Medihaler-Phen™ (phenylephrine-hydrocortisone-neomycin) for lasting, effective relief of nasal congestion.

Riker LOS ANGELES

clinically established*

FLEXIN®

Enteric coated • plain

in Low back pain

* SEE other side

flexin®

Zoxazolamine*

consistently effective in low back pain

"...Of 90 patients with low back pain and other muscular conditions... 67 (74 per cent) showed a good response...."[1]

"...17 of...20 patients with post-traumatic muscle spasm of the low back had excellent or good responses."[2]

"In acute and chronic recurrent low back syndrome, seven of eight patients showed visible objective improvement."[3]

Bibliography

(1) Johnson, H. J., Jr.: To be published. (2) Wallace, S. L.: To be published. (3) Settel, E.: Am. Pract. & Digest Treat. 8:443, 1957.

How Supplied

Pink, Enteric Coated tablets (250 mg.), bottles of 36.
Yellow, scored tablets (250 mg.), bottles of 50.

*U.S. Patent Pending

McNEIL

Laboratories, Inc.
Philadelphia 32, Pa.

11057

highly effective—clinically proved

Sigmamycin*

OLEANDOMYCIN TETRACYCLINE

provides added certainty in antibiotic therapy particularly for that 90% of the patient population treated in home or office...

Multi-spectrum synergistically strengthened SIGMAMYCIN provides the antimicrobial spectrum of tetracycline extended and potentiated with oleandomycin to include even those strains of staphylococci and certain other pathogens resistant to other antibiotics.

Supplied: **SIGMAMYCIN CAPSULES—250 mg. (oleandomycin 83 mg., tetracycline 167 mg.), bottles of 16 and 100; 100 mg. (oleandomycin 33 mg., tetracycline 67 mg.), bottles of 25 and 100. SIGMAMYCIN FOR ORAL SUSPENSION—1.5 Gm., 125 mg. per 5 cc. teaspoonful (oleandomycin 42 mg., tetracycline 83 mg.), mint flavored, bottles of 2 oz.**

***Trademark**

Pfizer

PFIZER LABORATORIES, Brooklyn 6, N. Y.
Division, Chas. Pfizer & Co., Inc.
World leader in antibiotic development and production

new

the logical combination for antibacterial

MYSTE

Squibb Tetracycline Phosphate Complex (Sumycin) + Nystatin (Mycostatin)

Each capsule contains tetracycline phosphate complex equivalent to 250 mg. tetracycline hydrochloride and 250,000 units Mycostatin.

Minimum adult dosage: 1 capsule q.i.d. Bottles of 16 and 100.

ALSO AVAILABLE:

Sumycin Capsules (tetracycline phosphate complex equivalent to 250 mg. tetracycline hydrochloride): Bottles of 16 and 100.

Mystecin Capsules (250 mg. tetracycline hydrochloride and 250,000 units Mycostatin): Bottles of 16 and 100.

Mystecin Half Strength Capsules (125 mg. tetracycline hydrochloride and 125,000 units Mycostatin): Bottles of 16 and 100.

Mystecin Suspension (fruit-flavored oil suspension containing equivalent of 125 mg. tetracycline hydrochloride and 125,000 units Mycostatin per 5 cc.): 2-ounce bottles.

SQUIBB *Squibb Quality—the Priceless Ingredient*

WHAT IS IT?

the phosphate complex
of tetracycline for initial
antibiotic blood levels...faster
and higher than ever before

+

antifungal activity of Mycostatin
for added protection against
monilial superinfection

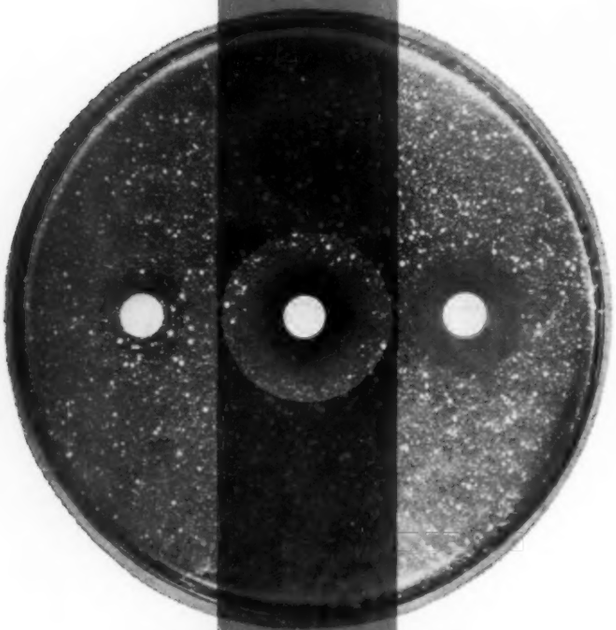
therapy and antifungal prophylaxis

CLINV

WHY SHOULD YOU PRESCRIBE IT?

Because it provides highly effective
broad spectrum antibiotic
therapy for many common infections
and at the same time protects
your patients against the monilial
overgrowth so commonly observed
during therapy with the
usual broad spectrum antibiotics

'MYSTECLIN'®, 'SUMYCIN' AND 'MYCOSTATIN'® ARE SQUIBB TRADEMARKS



Results on a mixed culture

of E. coli and Candida albicans: Panmycin* Phosphate (left) eliminates the bacteria, but not the monilia (white grains). Panmycin Phosphate and Nystatin combined (center) eliminate both the bacteria and monilia (clear ring). Nystatin (right) eliminates the monilia, but not the bacteria (haze).

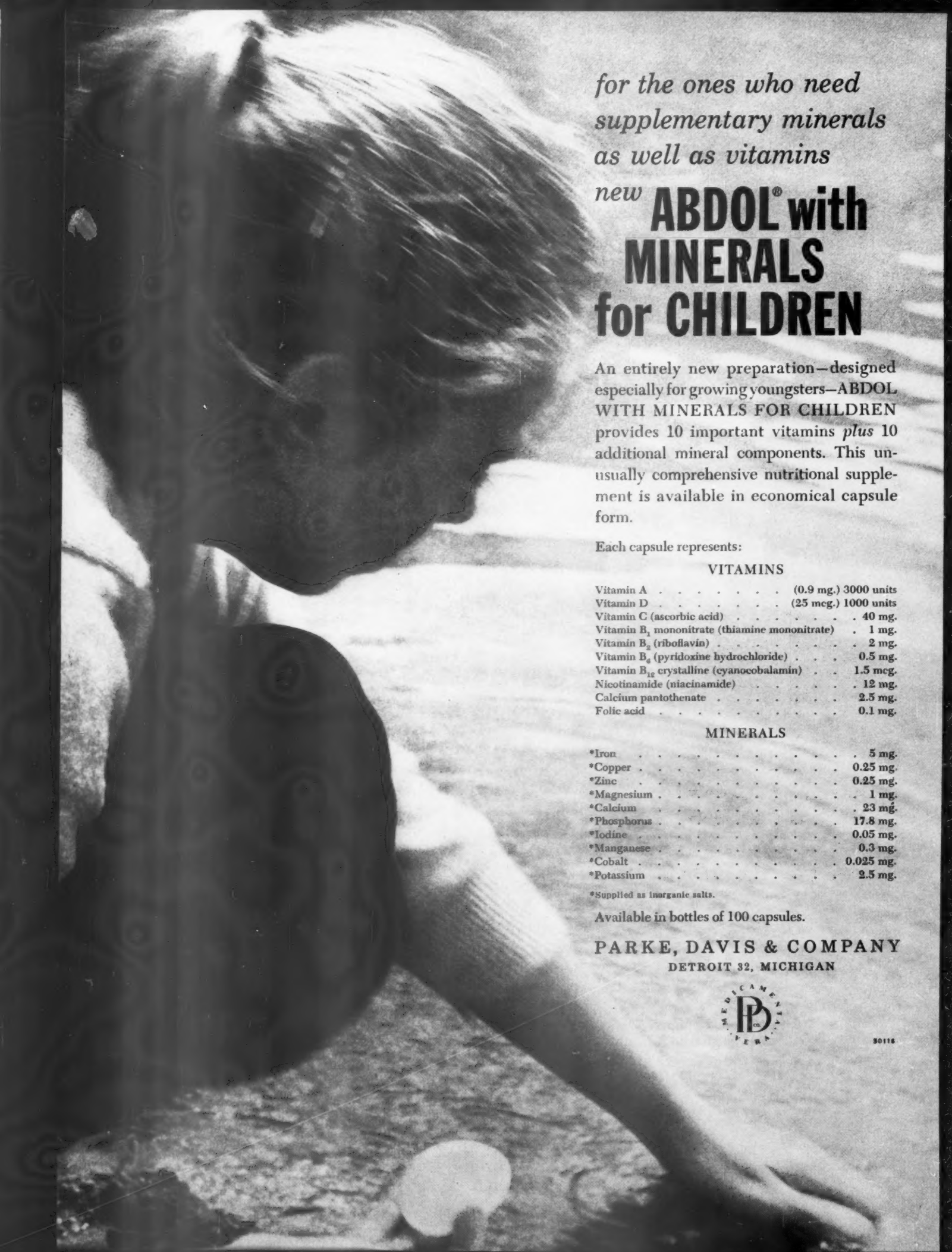
COMYCIN*

*TRADEMARK

A new salt of tetracycline, combined with Nystatin to protect against outgrowth of monilia

Each capsule contains: Tetracycline phosphate complex equivalent to tetracycline hydrochloride, 250 mg.; Nystatin, 250,000 units.
Indications and dosage: Same as for tetracycline HCl.
Supplied: Bottles of 16 and 100 capsules.
The Upjohn Company, Kalamazoo, Mich.

Upjohn



for the ones who need supplementary minerals as well as vitamins

new **ABDOL® with MINERALS for CHILDREN**

An entirely new preparation—designed especially for growing youngsters—ABDOL WITH MINERALS FOR CHILDREN provides 10 important vitamins *plus* 10 additional mineral components. This unusually comprehensive nutritional supplement is available in economical capsule form.

Each capsule represents:

VITAMINS

Vitamin A	(0.9 mg.)	3000 units
Vitamin D	(25 mcg.)	1000 units
Vitamin C (ascorbic acid)		40 mg.
Vitamin B_1 mononitrate (thiamine mononitrate)		1 mg.
Vitamin B_2 (riboflavin)		2 mg.
Vitamin B_6 (pyridoxine hydrochloride)		0.5 mg.
Vitamin B_{12} crystalline (cyanocobalamin)		1.5 mcg.
Nicotinamide (niacinamide)		12 mg.
Calcium pantothenate		2.5 mg.
Folic acid		0.1 mg.

MINERALS

*Iron	5 mg.
*Copper	0.25 mg.
*Zinc	0.25 mg.
*Magnesium	1 mg.
*Calcium	23 mg.
*Phosphorus	17.8 mg.
*Iodine	0.05 mg.
*Manganese	0.3 mg.
*Cobalt	0.025 mg.
*Potassium	2.5 mg.

*Supplied as inorganic salts.

Available in bottles of 100 capsules.

PARKE, DAVIS & COMPANY
DETROIT 32, MICHIGAN

MEDICAMENTA VERA

50116

"SUDDENLY I felt dizzy..."

Look out for the "little" strokes resulting from abnormal capillary fragility. Many cerebral accidents may be avoided if adequate amounts of capillary protective factors–hesperidin complex and ascorbic acid–are provided.[1]

The true character of the "little" strokes lies in their elusiveness—a sudden dizzy spell, temporary numbness of a hand, bizarre feeling of pain, or subtle personality change. Such symptoms are typical of little strokes and usually pass quickly, but they are likely to recur.[2,3]

Early recognition can gain vital therapeutic time. Hesper-C provides hesperidin complex and vitamin C essential for the protection of the capillaries to prevent further damage.

1. Gale, E. T., and Thewlis, M. W.: Geriatrics *8*:80, 1953. 2. Alvarez, W. C.: Geriatrics *10*:555, 1955. 3. Conference on Cerebral Vascular Disease, American Heart Association, Princeton, N. J., January, 1957.

Hesper-C

Available: As capsules—*and* NEW Hesper-C Liquid for your geriatric patients.

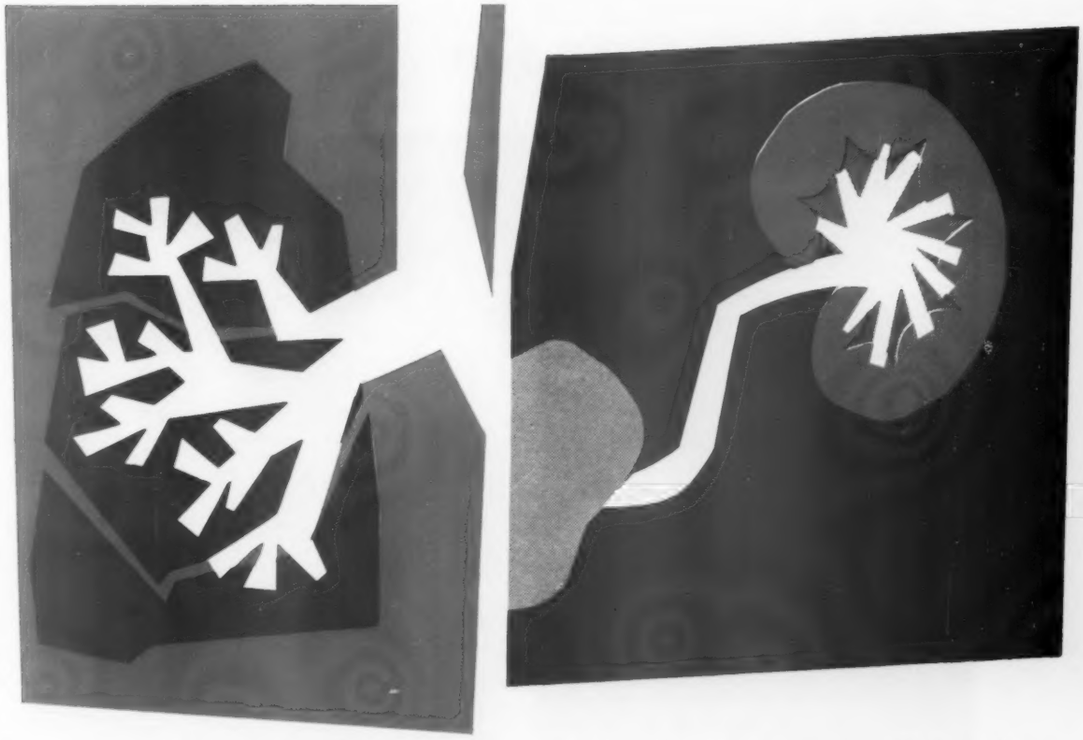
Provides: 100 mg. hesperidin complex plus 100 mg. ascorbic acid per capsule or teaspoonful (5 ml.) of syrup.

℞ 6 capsules or teaspoonfuls daily, or more. No toxicity or untoward effects have ever been reported even with massive doses.

Products of Original Research

THE NATIONAL DRUG COMPANY
Philadelphia 44, Pa.

H-1700/67



for prompt clinical response in many common infections
especially those of the urinary and respiratory tracts

prescribe **Terfonyl**

Squibb Triple Sulfas
Trisulfapyrimidines

- *Prompt, high blood levels*[1]
- *Excellent tissue diffusion*[1]
- *Highly soluble in the urine, especially at critical pH levels*[1]
- *Few sensitization reactions*[1]

Tablets, 0.5 Gm., bottles of 100 and 1000.

Raspberry-flavored Suspension, 0.5 Gm. per 5 cc. teaspoonful, pint bottles.

SQUIBB *Squibb Quality—the Priceless Ingredient*

"TERFONYL"® IS A SQUIBB TRADEMARK

1. Lehr, D.: Modern Med. 23:111 (Jan. 15) 1955.

TER-57-1

NEW...

Each Multiple Compressed Tablet of MEPROLONE provides the inseparable antiarthritic, antirheumatic benefits of:

1. *Prednisolone buffered*—the newest and most potent of the "predni-steroids" for prompt relief of joint pain and arrest of the destructive inflammatory process.

2. *Meprobamate*—the newest and safest of the muscle-relaxant tranquilizers for profound relaxation of skeletal muscle in spasm.

Tolerance to this combination is good because there is little likelihood of sodium retention, potassium depletion or gastric distress with buffered prednisolone, and meprobamate rarely produces significant side effects in therapeutic dosage.

An additional important therapeutic benefit, often overlooked, stems from the tranquilizing action of meprobamate. This component of MEPROLONE relieves mental tension and anxiety so often manifest in arthritics, making them more amenable to other rehabilitation measures.

INDICATIONS: A wide variety of conditions, in which four symptoms predominate: *a*) inflammation *b*) muscle spasm *c*) anxiety and tension *d*) discomfort and disability; i.e., rheumatoid arthritis, rheumatoid spondylitis (Marie-Strümpell disease), Still's disease, psoriatic arthritis, osteoarthritis, bursitis, synovitis, tenosynovitis, myositis, fibrositis, fibromyositis, neuritis, acute and chronic low back pain, acute and chronic primary and secondary fibrositis and torticollis, intractable asthma, respiratory allergies, allergic and inflammatory eye and skin disorders (as maintenance therapy in disseminated lupus erythematosus, periarteritis nodosa, dermatomyositis and scleroderma).

Therapeutic benefits of MEPROLONE compared with traditional antiarthritics.

	relieves pain	suppresses inflammation	relaxes muscle	eases anxiety	imparts sense of well-being
Salicylates	✓	✓			
Muscle relaxants			✓[1]		
Tranquilizers				✓[1]	
Steroids	✓	✓			✓
MEPROLONE	✓	✓	✓	✓	✓

1. Meprobamate is the only tranquilizer with muscle-relaxant action.

SUPPLIED: Multiple Compressed Tablets in bottles of 100 in two formulas as follows: MEPROLONE-1—1.0 mg. of prednisolone, 200 mg. of meprobamate and 200 mg. of dried aluminum hydroxide gel. MEPROLONE-2—provides 2.0 mg. of prednisolone in the same formula.

NO OTHER

ANTIRHEUMATIC

PRODUCT

PROVIDES AS MANY

BENEFITS AS

PROLONE'

MEPRO PREDNISO | BAMATE LONE, *buffered*

THE ONLY

ANTIRHEUMATIC,

ANTIARTHRITIC

THAT SIMULTANEOUSLY

RELIEVES:

1. MUSCLE SPASM
2. JOINT INFLAMMATION
3. ANXIETY AND TENSION
4. DISCOMFORT AND DISABILITY

MSD

MERCK SHARP & DOHME

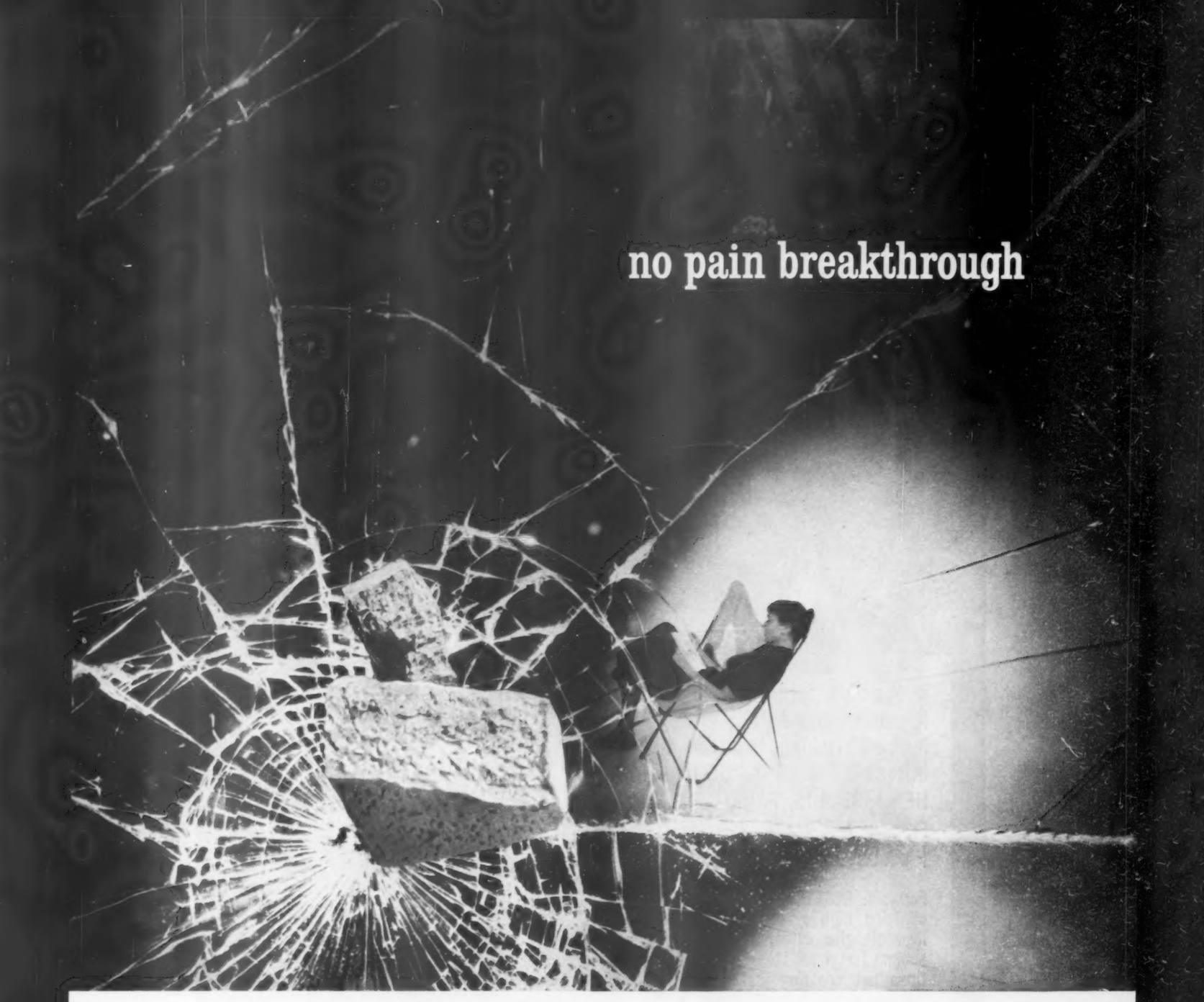
DIVISION OF MERCK & CO., INC. PHILADELPHIA 1, PA.

MEPROLONE is the trade-mark of Merck & Co., Inc.

check!

STERANE® *can't improve his gambit, help him castle or assure a checkmate... but* STERANE *can check asthmatic bronchospasm, dyspnea and wheezing to help your patient move about freely in almost any pastime or profession with minimum discomfort or restriction. Most potent corticoid,* STERANE *(prednisolone) is supplied as white, scored 5 mg. tablets (bottles of 20 and 100) and pink, scored 1 mg. tablets (bottles of 100).*

Pfizer PFIZER LABORATORIES *Division, Chas. Pfizer & Co., Inc. Brooklyn 6, New York*



One DONNAGESIC Extentab gives 10 to 12 hours of steady, high-level codeine analgesia. Rebuilding of effective analgesia with repeated doses is avoided. Patient comfort is continuous.

There is more pain relief in DONNAGESIC Extentabs than in codeine alone — codeine analgesia is potentiated by the phenobarbital present. In addition, phenobarbital diminishes anxiety, lowering patient's reactivity to pain.

DONNAGESIC is safer, too, for codeine side effects are minimized by the peripheral action of the belladonna alkaloids.

extended action—The intensity of effects smoothly sustained all-day or all-night by each DONNAGESIC Extentab is equivalent to, or greater than, the maximum which would be provided by q. 4h. administration of one-third the active ingredients.

Donnagesic™ Extentabs*

extended action tablets of CODEINE with DONNATAL®

once and for all every 10-12 hours codeine uses

	DONNAGESIC No. 1 (pink)	DONNAGESIC No. 2 (red)
CODEINE Phosphate	48.6 mg. (¾ gr.)	97.2 mg. (1½ gr.)
Hyoscyamine Sulfate	0.3111 mg.	0.3111 mg.
Atropine Sulfate	0.0582 mg.	0.0582 mg.
Hyoscine Hydrobromide	0.0195 mg.	0.0195 mg.
Phenobarbital	48.6 mg. (¾ gr.)	48.6 mg. (¾ gr.)

Robins **A. H. ROBINS CO., INC., RICHMOND, VIRGINIA** *Ethical Pharmaceuticals of Merit Since 1878*

*Reg. U. S. Pat. Off., Pat. applied for.

CONCLUSIONS:

after 2 years of extensive clinical use of...

RECTAL DESITIN® OINTMENT

"it has fulfilled better than any previously tried medicaments all the qualifications" expected of a proctologic ointment[1]

"promotes smooth epithelization and healthy granulation tissue and accelerates healing."[1]

- Specially formulated for prolonged, unusual efficacy in relieving pain, itching, irritation and inflammation in non-surgical HEMORRHOIDS, PRURITUS ANI, FISSURES, PERIANAL DERMATITIS, PAPILLITIS, etc. Non-sensitizing.

Formula: **RECTAL** DESITIN OINTMENT contains high grade Norwegian cod liver oil, zinc oxide, lanolin, talcum, sodium lauryl sulfate, petrolatum q.s. Does **not** contain local anesthetics, narcotics, or "caine" drugs which might mask serious anorectal disorders.

Available on your prescription in tubes of 1½ oz., with a safe, flexible applicator

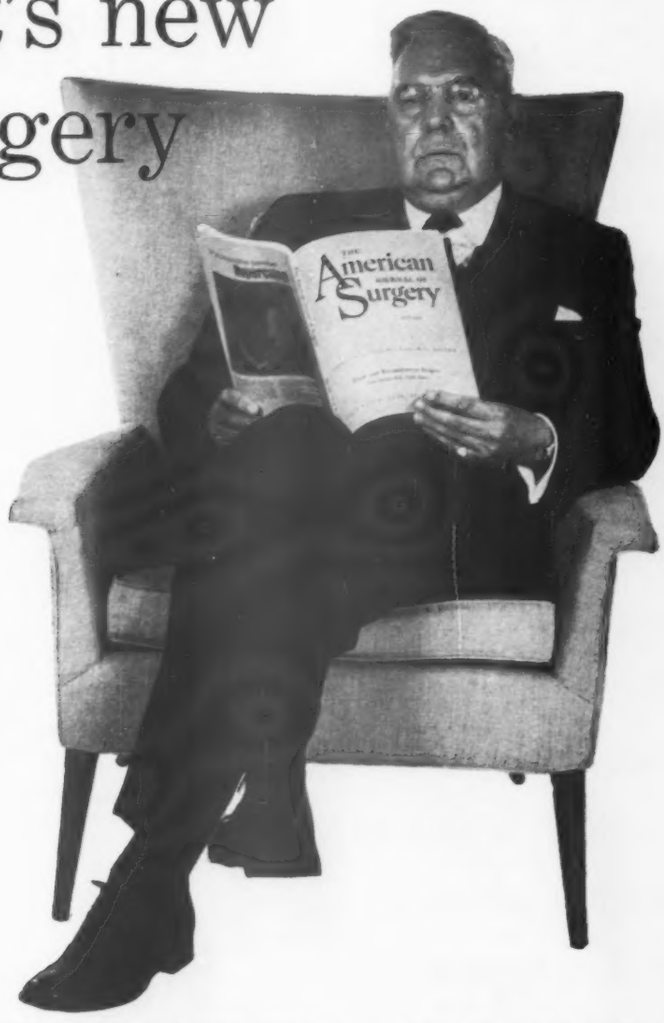
Liberal SAMPLE supply on request

DESITIN CHEMICAL COMPANY, PROVIDENCE 4, R. I.

New **RECTAL** DESITIN OINTMENT is not to be confused with regular DESITIN OINTMENT

1. Spiesman, M. G. and Malow, L.: Amer. J. Proctology, June 1956.

my first choice for what's new in surgery



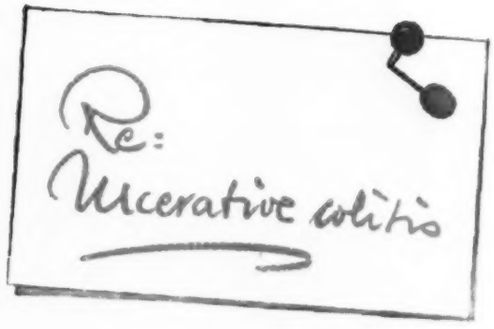
You, too, will find this authoritative, independent journal a *leader* for *first* reportings of what's new in general and specialized operative techniques. This practical journal presents regularly a treasury of skillfully executed illustrations, feature articles and important symposiums. Publishes the papers of Trauma, Maxillofacial and Pacific Coast societies. Edited by a board of internationally distinguished surgeons. Subscribe now, 12 months, U.S.A. $15.00, Canada $16.00, Foreign $17.00.

The American Journal of Surgery

OF THE YORKE GROUP

Publishers of The American Journal of Medicine, The American Journal of Clinical Nutrition, Modern Drugs.

49 West 45th Street, New York 36, N. Y.



This is "the most valuable drug that has been introduced for the treatment of ulcerative colitis" in recent years.[1] Results of treatment with Azulfidine "far exceed those of any previous drug used".[2] "It has been effective in controlling the disease in approximately two-thirds of patients who had previously failed to respond to standard colitis therapy currently in use."[3]

Azulfidine

BRAND OF SALICYLAZOSULFAPYRIDINE

1. Bargen, J. A.: "Present Status of Hormonal and Drug Therapy of Ulcerative Colitis", South. M. J. *48*: 192 (Feb.) 1955.
2. Bargen, J. A. and Kennedy, R. L. J.: "Chronic Ulcerative Colitis in Children", Postgrad. Med. *17*: 127 (Feb.) 1955.
3. Morrison, L. M.: "Response of Ulcerative Colitis to Therapy with Salicylazosulfapyridine", J. A. M. A. *151*: 366 (Jan. 31) 1953.

PHARMACIA LABORATORIES, INC.
501 Fifth Avenue, New York 17, N. Y.

increased strides

in rheumatoid arthritis

METICORTELONE®

prednisolone

...rapidly reduces swelling, tenderness and pain on motion

...overcomes disabling muscle spasm

...maintains therapeutic benefits by minimizing the sodium retention, edema and potassium loss associated with older corticosteroids

buff-colored tablets of 1, 2.5 and 5 mg.

Schering

ML-J-557

SAFETY FIRST

AFTER ALMOST FIVE YEARS OF INVESTIGATION AND EXTENSIVE CLINICAL USE (MILLIONS OF PRESCRIPTIONS) THERE HAS NOT BEEN A SINGLE REPORT OF A SERIOUS OR FATAL REACTION TO ERYTHROCIN

This remarkable safety record stands unparalleled in systemic antibiotic therapy today. In addition to being an unusually well-tolerated drug . . . ERYTHROCIN (compared to most other commonly-used antibiotics) is virtually free of side effects.

Still, with this virtual freedom from toxicity, ERYTHROCIN is effective in the great majority of common, bacterial respiratory infections. In speaking of pneumonia, Herrell said, *"the lack of toxic manifestations following administration of erythromycin today actually favors its use over that of the broad-spectrum antibiotics in the treatment of this infection."*[1]

While discussing purulent cellulitis and sepsis due to staphylococci, Eastman, et al., mentioned erythromycin as a *drug of first choice in treating these conditions.*[2]

Meanwhile, Solomon and Johnston stated, *"in the staphylococcic and streptococcic infections, other than pneumonias, without exception the results of treatment with erythromycin were excellent."*[3]

IN ANTIBIOTIC THERAPY

You, too, can have these same good results in your everyday practice—plus the assurance of prescribing a drug proved to be exceptionally well-tolerated in almost five years' use. *Filmtab* ERYTHROCIN Stearate (100 and 250 mg.), in bottles of 25 and 100.

FILMTAB®

Erythrocin®

STEARATE (Erythromycin Stearate, Abbott)

1. Herrell, W. E., Erythromycin, Antibiotics Monographs, No. 1, p. 34, New York, Medical Encyclopedia Inc., 1955. 2. Eastman, G., Cook, E. and Bunn, P., N. Y. State J. Med., 56:241, 1956. 3. Solomon, S. and Johnston, B., Amer. J. Med. Sc., 230:660, 1955.

Abbott

●Filmtab – Film-sealed tablets, Abbott; pat. applied for.

single sulfonamide specifically for urinary tract infections

direct / effective

"THIOSULFIL"

Brand of sulfamethizole

greater solubility means rapid action with minimum side effects

AYERST LABORATORIES
New York, N. Y. • Montreal, Canada
5652

Back Issues Wanted

(MUST BE IN GOOD CONDITION)

THE AMERICAN JOURNAL OF MEDICINE

will pay $1.00 per copy for the following issues:

January 1948

February 1948

May 1955

August 1955

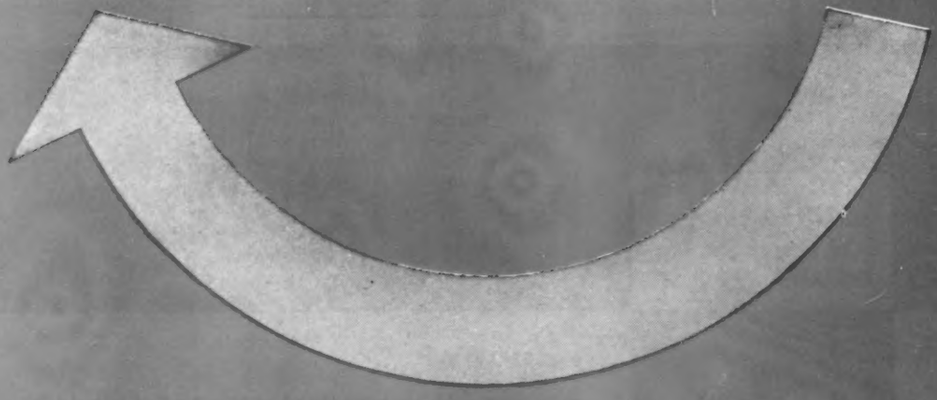
September 1955

October 1955

Send to

The American Journal of Medicine, Inc.
49 West 45th Street **New York 36, N. Y.**

prescribe RAUDIXIN to break the mental tension—hypertension cycle



*Raudixin reduces mental tension

Tranquilizing Raudixin reduces the mental tension which plays a significant role in hypertension . . . reduces mental tension as yet unrelated to physical symptoms.

*Raudixin reduces hypertension

Blood pressure lowering effect is gradual, sustained in hypertensives . . . little or no hypotensive effect is produced in normotensives.

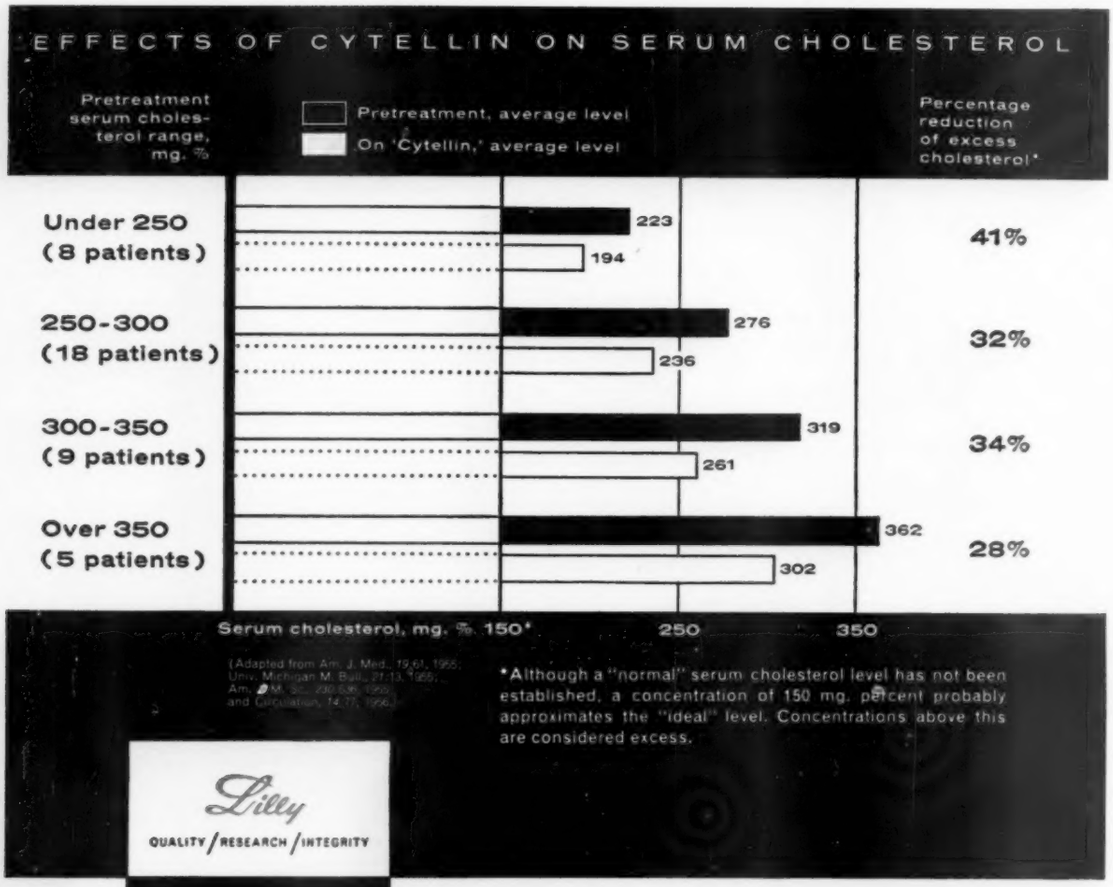
*Single daily dosage

Discourages promiscuous over-use by patients . . . not habit-forming.

RAUDIXIN

Squibb Whole Root *Rauwolfia Serpentina*

SQUIBB *Squibb Quality—the Priceless Ingredient*


EFFECTS OF CYTELLIN ON SERUM CHOLESTEROL
Pretreatment serum cholesterol range, mg. %
Pretreatment, average level
On 'Cytellin,' average level
Percentage reduction of excess cholesterol*
Under 250 (8 patients)
223
194
41%
250-300 (18 patients)
276
236
32%
300-350 (9 patients)
319
261
34%
Over 350 (5 patients)
362
302
28%
Serum cholesterol, mg. % 150* 250 350
(Adapted from Am. J. Med., 19:61, 1955; Univ. Michigan M. Bull., 21:13, 1955; Am. J. M. Sc., 230:636, 1955; and Circulation, 14:77, 1956.)
*Although a "normal" serum cholesterol level has not been established, a concentration of 150 mg. percent probably approximates the "ideal" level. Concentrations above this are considered excess.
Lilly
QUALITY / RESEARCH / INTEGRITY

reduces
elevated serum cholesterol

CYTELLIN

(Sitosterols, Lilly)

'Cytellin' reduces the absorption of dietary cholesterol and the reabsorption of endogenous cholesterol excreted in the bile. Severe dietary restrictions are not necessary to obtain a significant decline in serum cholesterol level. For a majority of patients, 'Cytellin' provides the most rational and practical therapy available.

In addition to lowering hypercholesteremia, 'Cytellin' has been reported to effect reductions in C/P ratio, S_f10-100 lipoproteins, and beta lipoproteins.

May we send more complete information?

ELI LILLY AND COMPANY • INDIANAPOLIS 6, INDIANA, U. S. A.

773027

The American Journal of Medicine

VOL. XXII MAY, 1957 No. 5

Foreword

IT is now nearly fifty years since Garrod [1] in his Croonian lectures reviewed his new concept of the "inborn error of metabolism," illustrating it by describing his preliminary studies in albinism, alkaptonuria, cystinuria and pentosuria. He made free use of the newly rediscovered Mendelian theory of inheritance and argued not only that some human beings were different from others on account of their genetic makeup (this difference being inherited according to the Mendelian laws), but also that such a hereditary difference between human beings could sometimes be mainly biochemical. He further suggested that the immediate mechanism for such apparent abnormalities might be the absence of an enzyme essential for normal metabolism. It will be noted that he anticipated, therefore, the current view of the close connection between genes and enzymes. It was also essential to his theory to postulate that such inborn errors of metabolism were lifelong constant features.

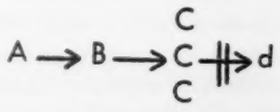
In reviewing our modern concept of these inborn errors, we need to modify only slightly Garrod's original views. First, however, let us attempt to define our present terms more closely. To this end I suggest the following: "By an inborn metabolic error is meant a permanent inherited condition due to a primary enzyme abnormality. As a result one or more chemical compounds may follow an altered metabolic pathway and may be found in some body fluids in greatly increased or decreased quantities." We must now consider further the more immediate results which could, theoretically at least, ensue from such a disorder.

$$A \rightarrow B \rightarrow C \rightarrow D$$

In the above diagram we are supposing that in the course of normal metabolism a substance A is changed successively by separate specific enzymes into substances B, C and D. If D is the final excretory product, say urea, carbon dioxide and the like, then it is likely to be formed somewhere in large quantities, but the preceding compounds, depending on their rate of turnover, need be present only in minute and perhaps undetectable amounts. If, now, a genetic defect occurs in which the enzyme for turning C into D is partly or completely absent (or for some reason inactive), clearly two consequences could ensue: (1) an accumulation of substance C which may appear to the casual observer as the presence of large quantities of a substance hitherto not suspected to occur in normal metabolism, and (2) an absence or reduced amount of the normal and well known metabolite D. It is also theoretically possible that in certain favorable situations the absence of the enzyme may be directly detectable by some assay method. This situation can be represented as follows:

Error of metabolism

or



Metabolic block

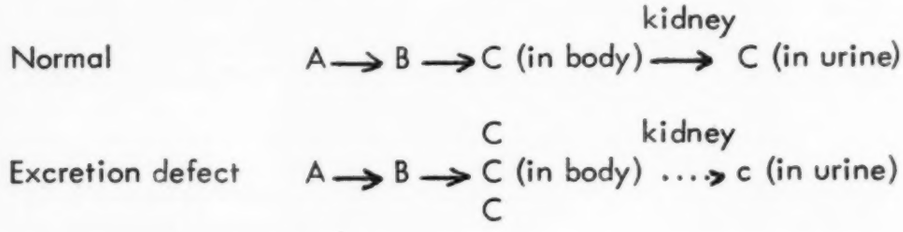
It is that envisaged by Garrod as accounting for some of the findings in albinism, alkaptonuria, cystinuria and pentosuria. There seems to be no doubt nowadays that this situation occurs in a number of cases. In albinism for instance (as Garrod also noted) it is the absence of a substance (melanin) rather than any accumulation which is the most obvious feature. In phenylketonuria it is the accumulation of phenylalanine, whose further metabolism is grossly defective, that points the way to the elucidation of the pathogenetic mechanisms. In hypophosphatasia we have a unique example where the first detected biochemical abnormality was the greatly diminished quantity of an enzyme (alkaline phosphatase). Clearly, when one or

[1] GARROD, A. E. The Croonian lectures on inborn errors of metabolism. *Lancet*, 2: 142, 1908.

another of these abnormalities is detected we at once get a lead as to what else we must look for. Indeed in many of the instances mentioned in this Symposium it will be noted how subsequent research has enable such further features to be discovered. We must stress, however, that it is not necessary for a primary enzyme absence or deficiency to show itself in such a gross manner, for there may well be alternative methods of producing and thus correcting the deficiency of the substance D or of disposing of a potential accumulation of substance C. It is no chance accident that many of the earlier discovered inborn errors have concerned disorders of aromatic substances. Presumably in such cases alternative methods of metabolism would more likely be absent and the inborn error thus more readily detectable. The situation would be more difficult to discover if it concerned smaller aliphatic molecules. For instance, an error of transmethylation from methionine to form choline might well lead to dire pathologic consequences. This, however, need not reveal itself by the accumulation of any appreciable amount of methionine, since methionine can be metabolised in more than one way and the amount normally used for transmethylation can thus be readily disposed of.

It is of interest that Garrod himself was greatly puzzled because his metabolic block theory could not explain many of the findings in human cystinuria. His theory necessarily implied that the substance which accumulated in body fluids was difficult for the body to metabolise. This certainly seemed to be the case in alkaptonuria when feeding homogentisic acid or its precursors led to large increases in the amounts of homogentisic acid excreted in the urine. Many of the earlier workers however had noted that this did not occur with cystine in cystinurics, for cystine given orally did not increase cystine output in the urine and seemed indeed to be readily metabolised in the normal manner. There was a further discrepancy in cystinuria, namely the presence in the urine in most cases of large quantities of the diamine bases cadaverine and putrescine, and also, in the opinion of a few workers, of other amino acids such as lysine, leucine and tyrosine. The unravelling of this particular problem is dealt with in detail later in this Symposium. It is sufficient to note here that a simple metabolic error in the Garrod sense should cause only simple biochemical abnormalities of the type mentioned in the foregoing diagrams and should not involve apparently unrelated chemical substances. We are forced, therefore, to introduce further concepts to understand these other findings.

The first of these is that of the excretion defect. This is very simple and merely means that a normal end product of metabolism which should be excreted in the urine will inevitably accumulate in the body if an abnormality of renal function with respect to this substance is present. The mechanism here can be shown as below:

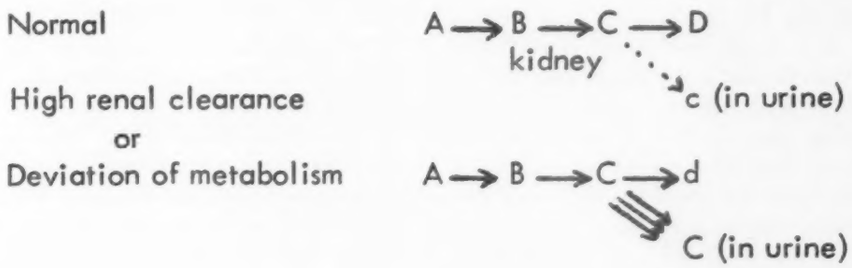


where the last stage is not a change of one substance into another but transportation of the same substance from one place to another. An example of this possible mechanism was mentioned by Garrod and concerned the accumulation of uric acid in patients with gout. Thannhauser [2] has recently upheld this interpretation although other workers in this field have made the defect appear much more complicated. This type of defect must remain a theoretic possibility for a limited number of compounds. Some workers have attributed the accumulation of cystine in cystine storage disease (cystinosis) to be due, as an immediate cause, to inability of the kidney to excrete cystine. The evidence is slender and in addition is difficult to accept in the case of a substance which is not a normal end product of metabolism.

A further possible mechanism also exists which was not suggested by any of the earlier workers. This is that a substance which is normally retained and effectively metabolised so that only minute quantities appear in the body

[2] THANNHAUSER, S. J. The pathogenesis of gout. *Metabolism*, 5: 582, 1956.

secretions may, however, appear in large quantities, say in the urine, owing to an abnormally increased excretion; that is, to a high renal clearance. This "renal" mechanism can be represented as follows:



This clearly occurs in renal glycosuria and it has always seemed puzzling to the present writer that this mechanism was not implicated until recently in cystinuria. Perhaps the concept was too physiologic for the biochemists who pioneered these fields! The renal mechanism has not even yet been fully excluded as the cause of the homogentisic aciduria occurring in alkaptonuria, nor in the more recently discovered xanthinuria [*3*] nor in the phosphoethanolaminuria occurring in hypophosphatasia. It will be seen later that such a renal mechanism involves essentially a transport process in which a compound is transferred without overall change in composition from one part of the organism to another. Even though such transport processes are under enzymic control they need not be so compound-specific as a true metabolic process (using this term in its more correct meaning which implies change of composition). The writer prefers to refer to this general type of leak mechanism as a "deviation of metabolism," implying that a compound which can be readily metabolised in the body is, so to speak, whisked away before this is allowed to happen. A different name is convenient, as this process is different in many ways from the classic Garrodian "error of metabolism." One would expect it, for instance, to be less harmful to the organism, and one would expect the quantitative relationship between the amount of the substance formed in the body and the amount recovered in the urine to be much less precise.

Finally there is a still further theoretic abnormality which could account for the superficial appearances of an inborn error of metabolism.

In this diagram it is assumed that the enzyme which in most people converts C into D is not absent nor present in lesser amount, but is replaced by an entirely different enzyme. This enzyme forms a different substance X, which therefore appears in the body as an accumulation of an apparently foreign substance. This mechanism, which might be termed a "metabolic freak," was not thought to be a very likely one when this subject was previously considered by the present writer [*4*]. He had in mind then that in many previous cases apparently freak substances of this kind (for instance, porphobilinogen) had eventually turned out to be normal metabolites. While this is probably still true for small molecules, it now seems certain that in the large structural proteins at any rate true freaks of this kind can occur. One need only mention for example the different forms of hemoglobin found in subjects with various forms of anemia.

It remains now to discuss the medical significance of these inborn errors of metabolism and of the various concepts that have been developed from and around them. The most fascinating and hopeful feature must already have been noted by any worker who has studied this group of diseases. This is that even apart from the family history their diagnosis is nearly always a comparatively simple matter because the inborn errors often represent wide deviations from normal. The difference between diagnosing a case of alkaptonuria, phenylketonuria or cystinuria and, for instance, a case of disseminated sclerosis, lupus erythematosus or pyelonephritis, is so great as to suggest that there is a completely different basic factor at work. This is perhaps the most happy aspect to be derived from the con-

[3] Dent, C. E. and Philpot, G. R. Xanthinuria. An inborn error (or deviation) of metabolism. *Lancet*, 1: 182, 1954.

[4] Dent, C. E. Der Aminosäurestoffwechsel. *Schweiz. med. Wchnschr.*, 80: 752, 1950.

cept of inborn errors for it suggests that the genetic theory may one day do for medicine what the atomic theory did for chemistry, namely, divide things up in a far more clear-cut manner, as if some of the differences between different people were "all or none." Arising from this, and assuming for the moment that genes behave as if they were indivisible particles of the nature of the chemist's atoms, we can further state as an act of faith, not yet proved nor ever capable of being proved, that in those cases in which hereditary diseases seem difficult to diagnose and which have a confusing clinical or biochemical picture, we clearly have not as yet gotten down to the basic chemical pathology, but are merely fiddling around with less important secondary phenomena. In such cases we are encouraged therefore to look further into the matter, hoping that once the primary gene action is known the question of diagnosis at least will from then on present no further problems. The writer is prepared to carry this argument still further and to state his belief that all hereditary diseases are due to basically biochemical disorders even when, as in arachnodactyly, diaphysial aclasis or achondroplasia, they manifest themselves by bizarre morphologic abnormalities in subjects otherwise enjoying good health. Likewise, it may be reasonable to hold as a first assumption that a disease which is homogeneous is therefore likely to be hereditary. It is indeed fortunate that the search for biochemical disorders in diseases of unknown cause has recently become much more feasible owing to the development of simple paper chromatographic technics. By means of a few carefully chosen chromatograms it is now possible to scan body fluids for abnormal concentrations of hundreds of known or likely metabolites.

When one studies the articles coming later in this Symposium one sees a further feature of these inborn errors, namely that they include examples from specialties in medicine as widely separate as neurology, pediatrics, dermatology, urogenital surgery, psychiatry and so forth. Indeed, if one surveys the whole group of inborn errors there is no branch of medicine which is not at some time or other implicated. We can therefore say that all doctors should know something about these diseases and of the principles involved. Furthermore, those who aim to specialise in genetics should try to avoid overspecialisation in medicine, especially by limiting themselves to any particular age group.

There is a further interesting feature which needs to be stressed with regard to the clinical manifestations of the metabolic errors. It is now apparent that even in cases in which the biochemical diagnosis is simple and can be made at any time of life, owing to its constant manifestation, the clinical picture may nevertheless vary from complete normality to acute attacks of disease, to long standing chronic disease, or to a disease which slowly develops at almost any time of life. This feature is well illustrated in the case of Hartnup disease (also called 'H' disease [5]). The reason for this variable clinical picture is sometimes obvious, for the genetic defect in many cases is not directly harmful but acts only to predispose to a disease which is actually due to an immediate cause in the environment. For instance, the genetic defect may produce a state of photosensitivity, or of abnormal sensitivity to some constant constituent of the diet such as copper, galactose, phenylalanine or vitamin D. Clearly one can suppose that in these cases the clinical manifestations must vary according to the nature of the environment, and one is entitled to argue backwards from this and to assume that when the clinical picture in any hereditary disease is variable one should look especially carefully for such environmental factors, for this should give one a lead for research and treatment in such cases. Once however one has admitted the importance of such environmental factors in hereditary disease, it is difficult to know where to stop, for it becomes probable that genetic influences may be present to a varying extent in almost any disease of any etiology.

The last point mentioned leads me to emphasize again the particular reason why the study of heredity is becoming of much greater relative importance in modern medicine. A hundred years ago environmental problems, such as those of crude nutrition and of epidemics of overwhelmingly infective diseases, were so important in themselves as to dominate almost entirely man's thinking for improvement of his health and standard of living. These problems had, naturally enough, led to the development of various materialistic social reforms and revolutions. The situation now seems entirely different, for these adverse environmental conditions have

[5] Baron, D. N., Dent, C. E., Harris, H., Hart, E. W. and Jepson, J. B. Hereditary pellagra-like skin rash with temporary cerebellar atopia, constant renal aminoaciduria and other bizarre biochemical features. *Lancet*, 2: 421, 1956.

been largely overcome in civilized countries. Nowadays the reasons why some people are healthier than others, are much more likely to be because they are different people with a different genetic make-up than because of their only slightly different and generally satisfactory environment. Seen in this light some of the mainly beneficial social movements of the past, having long fulfilled their purpose, now appear to be old fashioned, and their occasional antagonism to modern genetics [*6*], while easy to understand, seems rather pathetic.

One final point must be made now with regard to the question of organ involvement in these hereditary diseases. It is easy to state that in one disease a liver or bone enzyme is absent, or that in another disease there is a disorder in the renal tubule. In such cases, however, the genetic theory must be borne in mind again. If the diseases are caused by abnormal genes then the basic genetic abnormality must be present from the moment of conception, before any organ differentiation has occurred, and must later be passed on to every cell in the finally grown organism. Hence it is not correct to say that a certain hereditary disease is due to a primary defect of the renal tubule, while it is correct to say that it may first reveal itself as such an apparently isolated defect. There has been much theoretic confusion along these lines in the literature. This is also of practical importance in attempting to develop research programs. A defect which is readily detectable in the renal tubule should also be present, even if more difficult to detect, in every other body cell since all the cells contain the abnormal genes. The kidney may therefore be looked on as the "mirror of the metabolism of the whole organism," and, while continuing to delight ourselves in its ready accessibility, we should be encouraged nevertheless always to look for more generalized disorders, especially when the entire symptomatology does not already appear to be adequately explained. A good example of this is in cystinosis, when most of the patient's symptoms and biochemical findings are due to a renal tubule defect, and all one's therapy at the moment, which is temporarily very beneficial, is based on methods aimed to correct this defect. The most spectacular and quantitatively important finding however in this disease is that of the cystinosis, which represents in some organs a five hundred-fold increase of cystine content. So far we have no explanation for the cystinosis. It may be in some mysterious way caused by the renal tubule defect, but the genetic theory does not let us believe that this must be so. An excellent example of the deliberate search for and discovery of more generalised disorders in hereditary disease of apparently one organ is seen in galactosaemia, where it has been found that the red blood cells show a metabolic defect similar to that originally suspected in the liver (see later in this Symposium).

For the following Symposium, articles have been chosen which represent a fair sample of the various forms of inborn errors of metabolism. This field is becoming so large now that the choice has had to be limited. This has been done in order to include only diseases in which tangible biochemical abnormalities can be described and in which therefore some discussion can be developed as to the nature of the biochemical disorder. This has necessitated its limitation to metabolic errors involving enzymes which have relatively small molecules as substrates. We have therefore omitted any mention of the various interesting protein synthesis defects, such as those involving abnormal hemoglobins and plasma proteins.

CHARLES E. DENT, M.D.
University College Hospital Medical School
London, England

[6] LYSENKO, T. D. Soviet Biology. A report to the Lenin Academy of Agricultural Sciences, Moscow. London, 1948. Birch Books Ltd.

Symposium on Inborn Errors of Metabolism

Mechanisms in Genetic Diseases of Man*

VICTOR A. MCKUSICK, M.D.
Baltimore, Maryland

CURRENTLY, the genetic questions of most interest and importance to the clinician are those that concern mechanisms in diseases of primarily genic causation, the pathologic physiology of genetic disease. Three interrelated questions are involved: What is the "basic defect,"† that is, the deviation from the usual, responsible ultimately for the observed manifestation(s)? What is the *modus operandi* of the basic defect,† that is, what intermediate steps or links are involved in the production of the observed manifestation(s)? What factors account for the variations in severity of clinical expression and, in some instances, for failure of the disease to develop at all despite the presence of the appropriate mutant gene?

The reorientation of interest in genetic disease, as in the case of all hereditary traits, is indicated by the apt analogy used by Snyder [*3*] who, in surveying the status of human genetics at the mid-century, wrote as follows: "In the early days of the study of genetics, the geneticist was content to establish the correlation between the gene in the chromosome and the characteristic in the individual, without raising much question as to the chain of events which connected the two. The attitude was very much like that of the traveler who boards the Miami train in New York confident that he will step out at the Miami station, but actually knowing very little about the physico-chemical principles of the Diesel engine or the intricacies of the switching and signaling systems that will get him there.

Today, however, the question of genic action in the development of finished characters is uppermost in the minds of geneticists, and biochemical genetics is leading the attack on this front."

Obviously there are other matters of considerable importance to medical genetics. These include (1) devising methods for recognizing heterozygous carriers of recessively inherited disease;‡ (2) mapping the chromosomes, that is, identifying markers on chromosomes so that the linkage of disease traits can be used to predict the genetic state of a given individual in a given family in respect to a given disease; (3) calculation of risks for bearing affected children or of getting grave complications, if affected—for use in genetic counseling which presently is at best difficult; and (4) study of factors influencing mutation rate. From both the therapeutic and

† The adjective "basic" as used in this connection is a relative term. With one possible exception the precise derangement of the functionally inadequate molecule has in no instance been described. In familial periodic paralysis the demonstration of a loss of potassium from the serum to the muscle and in *adynamia episodica hereditaria* [*1*] a movement of potassium in the opposite direction has provided the first approximation to an explanation of these two hereditary states. However, there are obviously much more basic mechanisms involved. In osteogenesis imperfecta pin-pointing the defect to collagen was an important step. However, it is likely that even more precise identification of the biochemical aberration will be soon forthcoming. In sickle cell anemia an important beginning in the description of the exact structural peculiarity of the molecule of sickle hemoglobin has been made [*2*].

‡ For example, Hsia [*4*] has shown that presumably heterozygous parents of patients with the recessively inherited trait, phenylketonuria, have a mild or incomplete defect in the conversion of phenylalanine to tyrosine as revealed by phenylalanine tolerance tests. Similarly in diabetes insipidus, a defect in urinary concentrating power is identifiable in the presumably heterozygous parents [*5*].

* From the Department of Medicine, The Johns Hopkins University School of Medicine and The Johns Hopkins Hospital, Baltimore 5, Md.

the preventive point of view, these questions are not as important to the clinician, that is, to the physician concerned with the care of the individual patient, although they are matters of major concern to the field of public health.

In general, the main reasons for the importance of elucidating mechanisms might be listed as two. First, it is evident that treatment and prevention of disease in the individual patient must rest on a knowledge of mechanisms. This is a well accepted principle, which has unfortunately had thus far all too little confirmation in practice. It is a principle tacitly acknowledged by fund-granting agencies and special foundations for the study of specific heritable diseases. For example, sizable amounts of money are being spent to discover the "cause" of muscular dystrophy. It is, however, not the cause that is sought. The cause has long been known; the cause is a mutant gene, or rather, mutant genes, since a different gene is presumably responsible for each of the distinct types of hereditary muscular dystrophy. In actuality, what is sought are the mechanisms, the "basic defect," the modifying factors.

As indicated in this symposium, in phenylketonuria and in Wilson's hepatolenticular degeneration an intimate knowledge of mechanisms may permit effective treatment and prophylaxis in the not distant future. The simple expedient of withdrawing milk from the diet "cures" galactosemia. Furthermore, if the physician knew why the manifestations of genetic disease do not occur in some persons who carry the gene (why, as the geneticist puts it, the penetrance of the factor is reduced), if he knew why manifestations are negligibly mild in some persons (who, in the genetic jargon, display a low level of expressivity for the trait), then he might be able to mimic nature and influence the disease similarly by one maneuver or another. In hemochromatosis the female is partially protected from the disease through loss of blood and iron in the menses. Repeated venosections mimicking the physiologic process is effective treatment of the disease.

A second reason for the importance of studying genetic mechanisms is derived from the contribution to general biologic knowledge which can be made thereby. Agriculture and medicine are the two major divisions of applied biology. Probably in no connection does the physician have more frequent occasion to be reminded that he is basically a biologist than in relation to problems of inheritance. The relationship of medical genetics to general biology and its subdivisions is a two-way street. That medical genetics has much to learn from the biologic disciplines is obvious. Not so clearly recognized is the fact that clinical and allied investigation of genetic diseases can provide worth while and often otherwise unobtainable information about normal biologic processes. Sir Archibald E. Garrod, the acknowledged founder of biochemical genetics in man, enunciated this principle, illustrated and corroborated it by his own work, and further emphasized it [*6*] by the following quotation from a letter written by William Harvey in 1657, a few weeks before his death exactly 300 years ago:

"Nature is nowhere accustomed more openly to display her secret mysteries than in cases where she shows traces of her workings apart from the beaten path; nor is there any better way to advance the proper practice of medicine than to give our minds to the discovery of the usual law of Nature by careful investigation of cases of rarer forms of disease. For it has been found, in almost all things, that what they contain of useful or applicable nature is hardly perceived unless we are deprived of them, or they become deranged in some way."

The principle spelled out by Harvey and Garrod is perhaps nowhere better exemplified than in the case of the defects of the clotting mechanism. Eight or nine specific defects have been identified. These correspond to eight or nine specific essential factors in blood clotting which would be unknown were it not that patients have turned up with an hereditary deficiency of each. Whatever the field in which basic mechanisms are sought, hereditary perversions of the normal mechanisms should be studied: hereditary anomalies of renal tubular function should be helpful to the renal physiologist; hereditary gastrointestinal absorptive abnormalities to the gastroenterologist; inborn deviations in the function of endocrine glands to the endocrinologist.*

The search for the common denominator responsible for the diverse manifestations of hereditary syndromes is likely to result in new information of general biologic importance. It is certain to point up areas of ignorance. For example, investigation of fibrocystic disease of

* The work of Stanbury [*83*], demonstrating a different enzymatic defect in each of several varieties of goitrous cretinism, serves to illustrate this point.

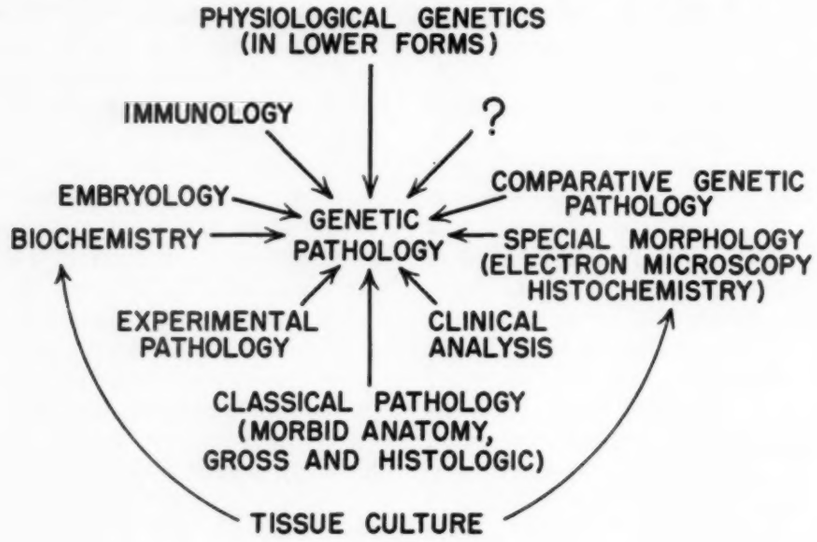


FIG. 1.

the pancreas and the manifold features not well indicated by this usual name of the syndrome—meconium ileus, biliary cirrhosis, bronchiectasis, pulmonary emphysema and fibrosis, "asymptomatic" changes in sweat and saliva—is likely to result in an increased knowledge of exocrine glands. "This type of thinking [relating diverse components of a syndrome] is bound to be profitable, if care is taken to distinguish hypothesis from proven fact, and to follow up promising hypotheses with pertinent evidence, observational and experimental" [7]. Jonathan Hutchinson, an indefatigable describer of syndromes, wrote: "We must analyse and seek to interpret, partnerships in disease" [*8*]. The blueprints for the analysis of mechanisms in hereditary diseases—the treasure maps, as it were—are the "pedigree of causes" diagrams which often begin to look a bit like the "flow chart" of a Rube Goldberg apparatus but which attempt to relate all manifestations of a given disorder back to a "basic" defect. The premises underlying such charts are (1) that all manifestations of a given syndrome are ultimately based on the action of a single gene (and are not dependent, for example, on gene linkage or some other mechanism), and (2) that "one-gene-one-function," if not a fundamental law of biology, is at least a satisfactory approximation. This is not the place to review the evidence for these well-founded tenets.

GENETIC PATHOLOGY

The problems under discussion here—problems in gene action—are subjects for investigation in the division of genetics called physiological genetics. Admittedly the whole category of hereditary disease-states merges imperceptibly at the edges into groups of "conditions" which if not considered entirely "normal" can scarcely be construed as diseases. I have in mind structural deviations such as polydactylism or accessory nipples. Nonetheless, physiological genetics seems an improper designation for this field of study when the trait requiring "explanation" is a disease. Genetic pathology seems more appropriate.

As is graphically represented in the accompanying diagram (Fig. 1) many disciplines have contributions to make to the field of genetic pathology. Furthermore, as mentioned earlier, feedback phenomena certainly occur. The question mark on the diagram is to indicate that it is entirely probable that important assistance in the solution of problems of genetic pathology may come from unexpected sources. We will comment on a few of the contributing areas specifically mentioned.

The availability of animals suffering from a genetic disease which is similar or possibly identical to human disease (comparative genetic pathology) should be helpful. Recently, for example, intense interest—and one might say optimism—has been stimulated by the report [*9*], from the Jackson Memorial Laboratory at Bar Harbor, of a mutation in mice which results in a disease resembling progressive muscular dystrophy of man in all important respects. The disease in mice seemingly identical with hereditary spherocytosis of man should give useful

TABLE 1

Disorder	Animal	Mode of Inheritance	Studied by*
Achondroplasia	Chick	Autosomal recessive	Lamoreux [29]
	Rabbit	Autosomal recessive	Pearce, Brown [30]
	Cattle	.....	Crew [31]
Albinism	Many birds and mammals	Autosomal recessive sex-linked recessive	Pearson et al. [32]
Anemia, ? type	Mouse	Autosomal recessive	Gowen, Gay [33]
Anemia, spherocytic hemolytic	Mouse	Autosomal recessive	Motulsky et al. [10, 87]
Anophthalmos	Mouse	.....	Chase, Chase [34]
Cystinuria	Dog	.....	Crane, Turner [35]
Dwarfism, ateliotic	Cattle, etc.	Autosomal recessive	Mead et al. [36]
Dwarfism, pituitary	Mouse	.....	Francis [37]
Ectopia lentis and cataract	Cattle	Autosomal recessive	Gregory et al. [38]
Hare-lip, cleft palate	Mouse	.....	Reed [39]
Hemophilia	Dog	Autosomal recessive	Hutt et al. [40]
Hypoglycemia	Mouse	Autosomal recessive	Cammidge, Howard [41]
Jaundice, hereditary	Rat	.....	Malloy, Lowenstein [42]
Myotonia congenita	Goat	.....	Kolb [43]
Obesity	Mouse	Autosomal recessive	Mayer [44]
Osteogenesis imperfecta (anosteoplasia congenita)	Cattle	.....	Inderbitzen [45]
Pelger anomaly of leucocyte	Rabbit	Autosomal recessive†	Nachtscheim [46]
Porphyria	Cattle	Autosomal recessive	Fourie [47]
Retinal degeneration	Rat	.....	Bourne et al. [48]
Syringomyelia	Rabbit	.....	Ostertag [49]

* —usually only reference to recent study given.
† —lethal homozygote.

information [10]. Table 1 presents a few of the animal counterparts of human disease which have been described to date. Surveys of the genetic traits which have been identified in domestic animals are available [11–21,84].

The herditary jaundice in the rat, described by Malloy and Lowenstein [42], has been shown to be due to defective conjugation of bilirubin to bilirubin glucuronide [85]. Calkins [86] has under study a disorder in French poodles which resembles osteogenesis imperfecta in man.

Tissue culture is potentially useful to genetic pathology in at least three ways. (1) Tissue culture can provide the biochemist with the pure cultures of cells which delight his heart and simplify his tasks. (2) It can help settle the question of whether a given disease is a primary and autonomous abnormality of one tissue or, alternatively, whether the pathologic changes observed in a given tissue are merely secondary to a remote metabolic, endocrine or other disorder. For instance, in muscular dystrophy the work of Geiger and Garvin [22] seems to have established that the primary defect, in the variety of the disease studied, resides in the muscle cell itself. In infantile genetic agranulocytosis, a recessively inherited condition identified in an isolate of northern Sweden [23], short-term cell culture, using bone marrow from affected subjects, has indicated that the basic defect is a disturbance of white blood cell maturation and that the defect can be corrected by addition of cysteine to the culture media. The last is a preview of another potential contribution of tissue culture. (3) The possibility of influencing the expression of the gene through changes in environmental factors can be explored *in vitro*. The experiments of Puck [24–26] illustrate what methodological progress has already been made in the application to mammalian cells growing in tissue culture of the same technics which have been used in studying biochemical genetics in bacteria and in molds, such as the classic Neurospora.

Experimental pathology can contribute to genetic pathology by the production of phenocopies, that is, facsimiles of the genetic disorder. The term "phenocopy" is Goldschmidt's [27]. Congenital malformations which in man are genetically determined can be closely simulated, in the experimental animal, by a large variety of maneuvers [28]. The toxin for connective tissue contained in the seed of the garden sweet pea (*Lathyrus odoratus*) produces kyphoscoliosis,

hernia and dissecting aneurysm of the aorta in rats—a syndrome resembling the gene-determined Marfan syndrome of man.

It is unnecessary to cite examples of the numerous contributions of the biochemists to genetic pathology, since the papers of this symposium provide adequate illustration. It is attractive to speculate that all genetic pathology will ultimately be reduced to biochemical terms.

There are a large number of hereditary states of developmental-morphologic type in which there is virtually no knowledge of the basic mechanism. Merely to select a few from among many, one might mention multiple exostoses, tuberose sclerosis, Osler-Rendu-Weber telangiectasia, Ollier's enchondromatosis, retinoblastoma, cystic disease of the kidneys, hypertrophic pyloric stenosis, the dysplasia of the acetabulum which is the structural substrate of congenital dislocation of the hip, brachyphalangy, achondroplasia. One suspects that embryology may have something to offer in the study of these disorders, especially in conjunction with biochemical and other technics.

Embryology has thus far contributed relatively little to a knowledge of mechanisms in genetic disease, mainly because the field has been handicapped by great technical difficulties. Embryogenesis is difficult to study in man. A comparison of embryogenesis in lower animals with special reference to specific malformations is likely to be helpful, as the experience [*50*] with cleft palate would appear to indicate. Chemical embryology, which attempts to define in chemical terms the phenomena of experimental embryology, can be expected to establish increasingly close connections with genetics.

Immunology, by defining differences between body proteins, should have useful contributions to make [*51*]. The globins of some abnormal hemoglobins are antigenically distinct [*52*]. Although there are other characteristics which more simply distinguish these particular abnormal proteins, there may be some disorders in which immunologic technics will be necessary.

The increasing importance of genetic disease, as other major categories such as nutritional and infectious are controlled, will necessitate a reorientation of educational curriculums not only for persons whose ambition it is to do research in the field of genetic pathology, but also for the medical student in general. Genetics and embryology will occupy an important place in such a reoriented medical curriculum.

THE COMPLEXITIES OF GENETIC PATHOLOGY

The complexities with which any analysis of genic action in man must cope become quickly evident. To begin with, man probably has 23* pairs of chromosomes, each possibly with as many as 5,000 genes. In the second place, there is rarely an immediate relationship between gene and phene. There is usually a chain (or better, a net) of reactions, each link of which is gene-controlled. Therefore, mutation in any one of several different genes may result in the same ultimate phenotype. Stern [*54*] uses the analogy to a stalled car: In this case the phene, stalling, can be due to a number of causes. Actually, as in the case of stalling, ancillary symptoms and the mode of inheritance may narrow down the possibilities as to the nature of the biochemical defect. Furthermore, the concept of alternative metabolic pathways [*56*] and that of competitive pathways open up many possibilities as to (1) variability in expression of a trait because of partial compensation through detour metabolic routes which may be open to varying degrees in different persons depending on other gene differences, and (2) production of the same phene by a number of different genes. A number of examples can be cited to illustrate the fact that many genes may have roughly similar effects: there appears to be more than one genetic variety of the adrenogenital syndrome [*57*]; although, as was pointed out to Garrod by Bateson soon after the rediscovery of Mendel's work, most cases of alcaptonuria demonstrate the pedigree characteristics of a recessive trait, there are occasional pedigrees [*58*] in which dominant inheritance is displayed and a slightly different biochemical lesion is possible although unproved. There is more than one mode of inheritance for albinism and for retinitis pigmentosa; there is probably a different gene responsible for each.

Parenthetically, however, it should be emphasized that current theories of dominance and recessivity [*59*] do not necessarily require one to conclude that a difference in mode of autosomal (non-sex-linked) inheritance necessarily indicates that a different gene is responsible for the given trait or a different link in the biochemical chain is defective, although probably as a rule such is the case. Further example: although con-

* Until recent studies [*53*] revealed the true situation, it has been thought and taught that man has 24 pairs of chromosomes.

genital spherocytic anemia is inherited as a dominant in man and as a recessive in the strain of mice studied by Motulsky [*87*], the difference in mode of inheritance is explicable on other grounds than different defect; the pathophysiology of the syndrome defect appears to be identical in the two species. One would expect, however, that a trait inherited in a sex-linked manner would be biochemically distinct from a phenotypically identical trait inherited autosomally in the same species.

The phenic consequences of genic action may be so remote from the primary operation of the gene that one may end up with the association of manifestations which are difficult to relate to each other. Such is the case in many hereditary syndromes. Elsewhere [*60*] the evidence for a single-gene basis of hereditary syndromes in man is presented. Accepting this seemingly adequately established hypothesis as fact, one is left with syndromes—melanin spots of the buccal mucosa, lips and digits associated with garden-variety adenomatous polyps of the gastrointestinal tract [*61*]; the syndrome of colonic polyps, osteomata and soft tissue tumors [*62*]; polycystic kidneys and intracranial aneurysm [*63*]; the association of obesity, retinitis pigmentosa, polydactylism, mental retardation and sexual infantilism in the Lawrence-Moon-Biedl syndrome;* retinitis pigmentosa with the Fanconi syndrome [*64*]; total color blindness with otosclerosis [*65*]; muscular dystrophy with baldness, myotonia, cataracts, testicular atrophy in Steinert's disease; and so on—in none of which current knowledge permits an understanding of the interrelationship of the component manifestations. Genuine pleiotropism, that is, multiple primary actions of a single gene, once considered a possible mechanism for the syndromal association of diverse phenes, now appears unlikely. Evidence from various sources suggests that each gene has but one primary action, the ramifications of which may, however, be exceedingly widespread. It is in the type of syndrome just cited by way of illustration that an analysis of the common denominator should reveal significant biologic information.

The complex interrelationships of genes can be looked upon as intracellular or intraorganismal ecology. The introduction of a goat [*55*] onto an oceanic island is likely to produce widespread effects; the relation of some of the consequences to the original change and to each other may not be immediately evident, nor may it be easy to sleuth out the basic defect responsible for radical changes in the physiognomy, fauna and flora of the island. The ecologic effects of the introduction of a mutant gene into a new genetic environment are similarly intricate.

Except for sex, the factors modifying the "expressivity" of mutant genes in man are largely unknown. Furthermore, in many instances the reason for sex differences* in the grade and frequency of inherited traits is poorly understood. In some situations the sex differences in expression are seemingly a result of the different hormonal environments. Hereditary hypercholesterolemia would appear to be an example. In general, women before the menopause have an advantage over men as to fat metabolism and consequently as to the incidence and severity of atherosclerosis. Castration of the female or the onset of menopause returns the female essentially to the position of the male in this regard. That estrogens are responsible for the sex differences in expression of the gene or genes responsible is a conclusion supported by experimental and clinical observations on the effects of administered estrogens. The effects of the gene for baldness may be potentiated by androgens such that heterozygous men are bald but women are bald only if homozygous for the trait (or heterozygous with a masculinizing tumor). In other instances differences in physiologic function, specifically the occurrence of menstruation and pregnancy in the female, account for the observed sex differences. For example, in hemochromatosis, the periodic loss of blood (and iron) in the menses provides a partially effective safety valve against development of the disease in women [*66*]. Physiologic differences in gametogenesis may be responsible for male-female differences in gout. Stetten [*67*] suggests that nucleoprotein metabolism associated with spermatogenesis is so much more active than that of oogenesis that the sex difference in expression of the gene for gout may be explicable on this

* The obesity and sexual infantilism may find explanation in a defect of nervous tissue which involves the hypothalamus and also involves the higher centers and retina, explaining mental deficiency and retinitis pigmentosa, respectively. The polydactylism is more obscure.

* Assumed in this discussion is that the sex difference is biological and not social or statistical. For example, rare biochemical defects, such as fructosuria, are sometimes apparently more frequent in men, probably only because more men than women are subjected to examination in connection with work, insurance and armed service.

basis. The *modus operandi* of the sex factor is unknown in many situations. Why does Marie-Strümpell spondylitis, which seems to be inherited as a dominant, occur in approximately 70 per cent of men with the gene but in only approximately 10 per cent of women? Why is hypertrophic pyloric stenosis of infants, quite clearly hereditary,* more frequent in males and, equally puzzling, in first-born males? Why is so-called congenital (actually hereditary) dislocation of the hip over five times more common in women [*69*]?

In general, the expression of a gene depends on the rest of the genetic makeup of the person and on factors of the environment, both intrauterine and extrauterine. The influence of the genetic milieu is sometimes observed in the Marfan syndrome: the characteristic skeletal proportions may be relatively unimpressive when the mutation occurs in pyknic stock. In the case of dominant conditions there is need for pedigree studies with analyses of the normal lines to determine the influence of various "normal" phenotypes on the expression of the mutant gene. To be decried is the usual practice in pedigree studies of concentrating on the side of the house through which the disease in question was inherited.

SOME PRINCIPLES OF GENETIC PATHOLOGY

Despite the complexities of genetic mechanisms a few principles have emerged; several of these have been outlined by Dent in the introduction to this symposium and are illustrated in the individual contributions. The following are also worthy of note:

The mechanism of genetically determined drug idiosyncracy has been identified in two instances. The first was the identification of the basis for susceptibility to hemolytic anemia from administration of certain agents [*70*]. It was found that the level of red cell glutathione is low and that the basic defect concerns glucose metabolism; specifically, glucose-6-phosphate dehydrogenase seems to be faulty [*71*]. The second is the susceptibility to prolonged, profound effects of succinyldicholine which is used as a muscle relaxant during anesthesia. The basis has been shown to be a deficiency of serum pseudocholinesterase, which under normal circumstances is responsible for the breakdown of the administered drug [*72*]. It is of note that in neither case would the genetic peculiarity become evident were it not for the administration of the drug. What is the basis for the not infrequently fatal idiosyncrasy of mongoloid idiots to agents of the atropine group? Genetic differences in susceptibility to infectious diseases such as tuberculosis [*73*] should eventually be explicable in terms analogous to these drug reactions [*74*]. Genetic differences in nutritional requirements and in susceptibility to allergens, thus far poorly understood, are further examples of the interplay of constitution and environment.

There are probably many hereditary diseases in which, in the last analysis, all the clinical manifestations stem from the fact that a given species of protein molecule is fabricated in a manner that places it at a functional disadvantage. This is what Pauling and his associates term "molecular disease" [*75,76*]. The best-studied example is sickle cell disease. Some subtle abnormality of the globin of hemoglobin [*2*] results in striking functional differences which are responsible for the manifestations of this disease. Abnormal protein synthesis is almost certainly at the basis of many diseases: abnormal fibrous proteins in heritable disorders of connective tissue,* abnormal contractile protein in muscular dystrophy,* and so on.

Furthermore, in diseases which have been discussed or referred to in this symposium and in which the basic mechanism is thought of as being an "absent" enzyme of intermediary metabolism, "absent" enzymes of specific functional significance as in the renal tubule, "absent" proteins with special function as in clotting or immunologic reactions, and the like, may all be somewhat artificial distinctions among members of a general group of conditions in which proteins are present, that is, normal quantitatively, but defective in some functional respect because of a structural peculiarity. It is entirely probable that in most if not all of the instances described the specific protein to which the defect is related is present but it has lost the earmarks by which we identify it. In the cases of many proteins, the enzymes for instance, function is the only criterion of identification. In this connection Pauling's concept of molecular disease is, as he

* Carter and Powell [*68*] made an extensive investigation which principally involved tracing infants who survived the Rammstedt operation 1920–1929 and who by the time of the study (published in 1954) had children of their own. Of 197 second-generation children, nine boys and one girl had had the Rammstedt operation!

* These are obviously speculations, the special license of the essayist.

maintains [*76*], of general significance. The possibilities for human variation and for disease on a genetic basis and through the agency of molecular changes are indicated by Pauling's statement that "there are of the order of 100,000 different kinds of proteins in the human body." One is impressed with the great importance of protein synthesis in genetic disease; the machinery for protein synthesis must be linked very closely to the gene itself.

In connection with the Seabright bantam cock, which has a comb with female characteristics despite the obvious virility of the bird, there has been defined a principle applicable in several areas of human genetic pathology: the defect in a disease which has the phenotypic pattern of a hormone deficiency may reside in the receptor organ which is genetically incompetent to respond to a normal amount of circulating hormone. Examples are represented by pseudohypoparathyroidism [*82*], nephrogenic diabetes insipidus, possibly ateliotic dwarfism.

The fascinating recent work on the pathogenesis of congenital spherocytic anemia [*77*] possibly illustrates a significant principle, namely, that it is possible to identify a biochemical basis for anomalous structure. It would have been easy a decade ago to accept this condition merely as a hereditary malformation of the erythrocyte (which in a sense it is) which renders it unusually fragile to osmotic and mechanical stress and susceptible to destruction in the spleen. It now seems likely that there is a defect in the glycolytic process which provides energy for maintenance of the integrity of the red cell stroma and membrane. Autohemolysis after trapping of red cells and depletion of substrate in the spleen is probably mainly responsible for the anemia. The biochemical basis for other structural abnormalities must be sought.

If not a principle of gene action, a useful concept is that implicit in the term *abiotrophy* invented by Gowers [*78*], who described the phenomenon in connection with certain neurologic disorders: that tissues of the central nervous system are clinically normal and probably histologically normal (by available methods of examination) in early life, but have a subtle innate weakness which results in their premature degeneration functionally and structurally. Several mechanisms for a picture of abiotrophy are possible. The mechanism to which the term is possibly most legitimately applied is that seemingly operative in the skin in pseudoxanthoma elasticum and in the aorta in the Marfan syndrome [*60*]. Both structures probably are at birth normal by all available criteria but do have a defect, probably of collagen in the first example, possibly of the elastic fiber in the second, which renders them liable to breakdown with the usual stress and strain. The skin of the flexural folds is first to show changes in pseudoxanthoma elasticum, whereas the ascending aorta which is subjected to maximal hemodynamic stress of particular types demonstrates the earliest and predominant changes in the Marfan syndrome. A mechanism for what might be called pseudoabiotrophy is the accumulation of an unidentified material which is toxic to a specific tissue or cell type. Until such time as the pathogenesis is worked out such a disorder might be misinterpreted as an abiotrophy. Wilson's hepatolenticular degeneration is an example of pseudoabiotrophy.

I think abiotrophy is a useful concept even if for no other reason than that it points up the fact that a disease can be primarily hereditary in etiology even though at birth it is not present by any current methods of detection applied to the individual. The concept can be a deterrent to progress if it is not appreciated that the basic defect *is* present at birth and will eventually probably be identifiable even in its preclinical state.

Another phenomenon concerning gene action is the conditioning of specific congenital malformations as the result of the presence during embryogenesis of a specific defect. Examples [*60*] seem to be provided in the Marfan syndrome and the Ehlers-Danlos syndrome, which appear to be specific defects of specific elements of connective tissue. However, in these syndromes certain congenital malformations occur with increased frequency and probably result from effects of the weakness of connective tissue at vital points in embryologic development.

A now familiar, though relatively recently discovered, general mechanism of genetic pathology concerns fetal-maternal interrelationships, specifically the immunologic effects of certain genetic differences between mother and fetus. Rh incompatibilities are the major known examples, but others, such as in the ABO series, are known and still others are probably as yet undiscovered.

In breast cancer of mice the milk factor which is responsible for an apparent hereditary nature behaves like a virus [*79*]. That a comparable

situation in man exists in the case of colonic polyps is suggested by the description of cytoplasmic inclusions which have morphologic characteristics of viral inclusion [*40,81*]. Admittedly these are not genetic diseases in the precise sense. Cytoplasmic inheritance is an alternative possibility raised by this finding of DNA-like material.

COMMENTS

Obviously, all diseases are influenced in at least some small part by genetic factors. Each sick person is a different, complex organization of genes who because of these differences has a particular susceptibility to a given disorder and a particular mode of reaction to disease. This is why we tell students there is no such thing as a "negative family history"— as absurd as recording "no spleen" in the normal physical examination. Genetic mechanisms in disease which are not primarily caused by a mutant gene are also important to elucidate, for example tuberculosis, as has already been mentioned.

Furthermore, just as heredity can influence diseases primarily of environmental origin, environment can influence genetic diseases. This fact is used in the treatment of genetic disorders. In coronary artery disease both a hereditary factor, in the form of peculiar fat metabolism, and diet, particularly increased fat ingestion, may be factors. Depending on its character, diet can favor or inhibit expression of the disease. It is difficult to know whether coronary artery disease should be classified as predominantly environmental or genetic, but the two certainly are not mutually exclusive.

I have attempted to show that, although the ultimate goal, and a reasonable one, is definition of the biochemical nature of genetic diseases, a general view of mechanisms, not confined to the biochemical aspects, in genetic diseases of man can and should be taken.

It has often seemed entertaining to imagine, Jules Verne style, bizarre diseases previously unknown. If one were to do this, imagining hereditary defects and basing the speculations on a sound knowledge of normal mechanisms, one could predict with considerable assurance that the imagined disease would at some time be encountered.

Acknowledgment: I am indebted to my colleagues, Drs. Barton Childs and Bentley Glass, for helpful criticisms.

REFERENCES

1. Gamstorp, I. Adynamia episodica hereditaria. *Acta Paediat.*, 45: supp. 108, 1956.
2. Ingram, V. M. A specific chemical difference between the globins of normal human and sickle-cell anaemia haemoglobin. *Nature*, 178: 792, 1956.
3. Snyder, L. H. Frontiers in genetics. In: Frontiers in Medicine: The March of Medicine, 1950, p. 56. New York, 1951. Columbia University Press.
4. Hsia, D. Yi-Yung. Detection by phenylalanine tolerance tests of heterozygous carriers in phenylketonuria. *Nature*, 178: 1239, 1956.
5. Carter, C. and Simpkiss, M. The "carrier" state in nephrogenic diabetes insipidus. *Lancet*, 2: 1069, 1956.
6. Garrod, A. E. The lessons of rare maladies. *Lancet*, 1: 1055, 1928.
7. McKusick, V. A. The new look in medical genetics. *Clin. Res. Proc.*, 3: 187, 1955.
8. Hutchinson, J. *Arch. Surg.*, 4: 361, 1893.
9. Michelson, A. M., Russell, E. S. and Harman, P. J. Dystrophia muscularis: a hereditary primary myopathy in the house mouse. *Proc. Nat. Acad. Sc.*, 41: 1079, 1955.
10. Motulsky, A. G., Huestis, R. and Anderson, R. Hereditary spherocytosis in mouse and man. *Acta Genetica et Statistica Medica*, 6: 240, 1956.
11. Hutt, F. B. Genetics of the Fowl. New York, 1949. McGraw-Hill Book Co., Inc.
12. Levi, W. M. The Pigeon. Columbia, 1941. R. L. Bryan Co.
13. Grüneberg, H. The Genetics of the Mouse, 2nd ed. Edited by Nijhoff, The Hague. 1952.
14. Bamber, R. C. Genetics of Domestic Cats. In: Bibliographia Genetica, vol. 3. Edited by Nijhoff, The Hague. 1927.
15. Burns, M. The Genetics of the Dog. Commonwealth Agricultural Bureau, Farnham Royal, Slough Bucks, England, 1952.
16. Roberts, J. A. F. and Crew, F. A. E. The Genetics of the Sheep. In: Bibliographia Genetica, vol. 2, The Hague. 1925.
17. Smith, A. D. B., Robinson, O. J. and Bryant, D. M. The Genetics of the Pig. In: Bibliographia Genetica, vol. 12, The Hague. 1936.
18. Berge, S. The hereditary anomalies in pigs. *Hereditas*, 27: 176, 1941.
19. Crew, F. A. E. and Smith, A. D. B. The Genetics of the Horse. In: Bibliographia Genetica, vol. 6, The Hague. 1930.
20. Shrode, R. R. and Lush, J. L. The genetics of cattle. *Adv. Genetics*, 1: 209, 1947.
21. Sawin, P. B. Recent genetics of the domestic rabbit. *Adv. Genetics*, 7: 183, 1955.
22. Geiger, R. S. and Garvin, S. J. Growth pattern in vitro of skeletal muscle from pseudohypertrophic muscular dystrophy. *Fed. Proc.*, 14: 55, 1955.
23. Kostmann, R. Infantile genetic agranulocytosis (agranulocytosis infantilis hereditaria). A new recessive lethal disease in man. *Acta pediat.*, 45: 1–78, 1956.
24. Puck, T. T., Marcus, R. I. and Cieciura, S. Clonal growth of mammalian cells *in vitro*. Growth characteristics of colonies from singe HeLa cells with and without a "feeder" layer. *J. Exper. Med.*, 103: 273, 1956.

25. Puck, T. T. and Fisher, H. W. Genetics of somatic mammalian cells. I. Demonstration of the existence of mutants with different growth requirements in a human cancer cell strain (HeLa). *Ibid.*, 103: 427, 1956.
26. Puck, T. T. and Marcus, P. I. Action of x-rays on mammalian cells. *Ibid.*, 103: 653, 1956.
27. Goldschmidt, R. B. Physiological Genetics. New York, 1938. McGraw-Hill Co.
28. Warkany, J. Congenital malformations induced by maternal dietary deficiency. *Harvey Lectures*, 48: 89, 1952–1953.
29. Lamoreux, W. F. Hereditary chondrodystrophy in the fowl. *J. Hered.*, 33: 275, 1942.
30. Pearce, L. and Brown, W. Hereditary achondroplasia in the rabbit. *J. Exper. Med.*, 82: 241, 261, 281, 1945.
31. Crew, F. A. E. The significance of an achondroplasia-like condition met with in cattle. *Proc. Roy. Soc.*, 95: 228, 1923.
32. Pearson, K., Nettleship, E. and Usher, C. H. A Monograph on Albinism in Man. Part II. Text and Atlas. London, 1913. Cambridge University Press.
33. Gowen, J. W. and Gay, E. H. Physiological factors necessary to alleviate genetic lethal anemia in mice. *Am. Nat.*, 66: 289, 1932.
34. Chase, H. B. and Chase, E. B. Studies on an anophthalmic strain of mice. I. Embryology of the eye region. *J. Morph.*, 68: 279, 1941.
35. Crane, G. W. and Turner, A. W. Amino acid patterns of urine and blood plasma in a cystinuric Labrador dog. *Nature*, 177: 237, 1956.
36. Mead, S. W., Gregory, P. W. and Regan, W. M. Proportionate dwarfism in Jersey cows. *J. Hered.*, 33: 411, 1942.
37. Francis, T. The development of the pituitary at hereditary anterior pituitary dwarfism in mice. *Op. Dom. Biol. hered. hum.*, vol. 7, Copenhagen, 1944.
38. Gregory, P. W., Mead, S. W. and Regan, W. M. A congenital eye defect in cattle. *J. Hered.*, 34: 125, 1943.
39. Reed, S. C. Harelip in the house mouse. *Genetics*, 21: 339, 1936.
40. Hutt, F. B., Richard, C. G. and Field, R. A. Sex-linked hemophilia in dogs. *J. Hered.*, 39: 3, 1948.
41. Cammidge, P. J. and Howard, H. The hereditary transmission of hypoglycemia in mice. *Proc. Roy. Soc. Med.*, 23: 1341, 1937.
42. Malloy, H. T. and Lowenstein, L. Hereditary jaundice in the rat. *Canad. M. A. J.*, 42: 122, 1940.
43. Kolb, L. C. Congenital myotonia in goats. *Bull. Johns Hopkins Hosp.*, 63: 221, 1938.
44. Mayer, J., Russell, R. E., Bates, M. W. and Dickie, M. M. Metabolic, nutritional and endocrine studies of hereditary obesity-diabetes syndrome of mice and mechanism of its development. *Metabolism*, 2: 9, 1953.
45. Inderbitzin, A. Ueber Anosteoplasia congenita beim Kalbe. *Virchows Arch. f. path. Anat.*, 269: 665, 1928.
46. Nachtsheim, H. The Pelger anomaly in man and rabbit. A mendelian character of the nuclei of the leucocytes. *J. Hered.*, 41: 131, 1950.
47. Fourie, P. J. J. Bovine congenital porphrinuria (pink tooth) inherited as a recessive character. *Onderstepoort J. Vet. Sc.*, 13: 383, 1939.
48. Bourne, M. C., Campbell, D. A. and Tansley, K. Hereditary degeneration of the rat retina. *Brit. J. Ophthal.*, 22: 613, 1938.
49. Ostertag, B. Weitere Untersuchungen über vererbbare Syringomyelie des Kaninchens. *Deutsch. Ztschr. Nervenheilk*, 116: 147, 1930.
50. Fraser, F. C. Etiological factors in clefts of the palate and lip. Paper presented at the First International Congress of Human Genetics, Copenhagen, 1956.
51. Irwin, M. R. Immunogenetics. *Adv. Genetics*, 1: 133, 1947.
52. Goodman, M. and Campbell, D. H. Differences in antigenic specificity of human normal adult, fetal, and sickle-cell anemia hemoglobin. *Blood*, 8: 422, 1953.
53. Ford, C. E. and Hamerton, J. L. The chromosomes of man. *Nature*, 178: 1020, 1956.
54. Stern, C. Principles of Human Genetics. San Francisco, 1950. W. H. Freeman & Co.
55. Stern, C. Gene and character. In: Genetics, Paleontology and Evolution. Edited by Lepsen, G. L., Mayr, E. and Simpson, G. G. Princeton, 1949. Princeton University Press.
56. Potter, V. R. and Heidelberger, C. Alternative metabolic pathways. *Physiol. Rev.*, 30: 487, 1950.
57. Childs, B., Grumbach, M. M. and Van Wyk. J. J. Virilizing adrenal hyperplasia: a genetic and hormonal study. *J. Clin. Investigation*, 35: 213, 1956.
58. Milch, R. A. Direct inheritance of alcaptonuria. *Metabolism*, 4: 513, 1955.
59. Wright, S. Physiological and evolutionary theories of dominance. *Am. Nat.*, 68: 24, 1934.
60. McKusick, V. A. Heritable Disorders of Connective Tissue. St. Louis, 1956. C. V. Mosby, Co.
61. Jeghers, H., McKusick, V. A. and Katz, D. H. Generalized intestinal polyposis and melanin spots of the oral mucosa, lips and digits. *New England J. Med.*, 241: 993, 1031, 1949.
62. Plenk, H. P. and Gardner, E. J. Osteomatosis (leontiasis ossea). Hereditary disease of membranous bone formation associated in one family with polyposis of the colon. *Radiology*, 62: 830, 1954.
63. Poutasse, E. F., Gardner, W. J. and McCormack, S. J. Polycystic kidney disease and intracranial aneurysm. *J. A. M. A.*, 154: 741, 1954.
64. Jackson, W. P. V. and Linder, G. C. Innate functional defects of renal tubules, with particular reference to Fanconi syndrome: cases with retinitis pigmentosa. *Quart. J. Med.*, 22: 133, 1953.
65. MacGregor, A. G. and Harrison, R. Congenital total color blindness associated with otosclerosis. *Ann. Eugenics*, 15: 219, 1950–1951.
66. Marble, A. and Bailey, C. C. Hemochromatosis. *Am. J. Med.*, 11: 590, 1951.
67. Stetten, DeW., Jr. Personal communication, 1956.
68. Carter, C. O. and Powell, B. W. Two-generation pyloric stenosis. *Lancet*, 266: 746, 1954.
69. Idelberger, K. Die Erbpathologie der sogenannter angeborenen Hüftverrenkung. Munich, 1951. Urban & Schwarzenberg.
70. Dern, R. J., Weinstein, I. M., LeRoy, G. V., Talmadge, D. W. and Alving, A. S. The hemolytic effect of primaquine. I. The localization of the drug-induced hemolytic defect in primaquine

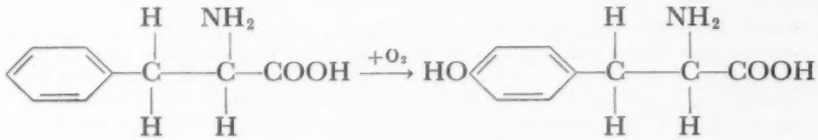
sensitive individuals. *J. Lab. & Clin. Med.*, 43: 303, 1954.
71. Carson, P. E., Flanagan, C. L., Ickes, C. E. and Alving, A. S. Enzymatic deficiency in primaquine-sensitive erythrocytes. *Science*, 124: 484, 1956.
72. Lehmann, H. and Ryan, E. The familial incidence of low pseudocholinesterase level. *Lancet*, 2: 124, 1956.
73. Kallman, F. J. and Reisner, D. Twin studies on genetic variations in resistance to tuberculosis. *J. Hered.*, 34: 269, 293, 1943.
74. Lurie, M. B., Zappasodi, P., Dannenberg, A. M., Jr. and Weiss, G. H. On the mechanism of genetic resistance to tuberculosis and its mode of inheritance. *Am. J. Hum. Genetics*, 4: 302, 1952.
75. Pauling, L., Itano, H. A., Singer, S. J. and Wells, I. C. Sickle-cell anemia, a molecular disease. *Science*, 110: 543, 1949.
76. Pauling, L. Abnormality of hemoglobin molecules in hereditary hemolytic anemias. *Harvey Lecture*, 49: 216, 1953–1954.
77. Young, L. E. Hereditary spherocytosis. *Am. J. Med.*, 18: 486, 1955.
78. Gowers, W. R. Abiotrophy. *Lancet*, 1: 1003, 1902.
79. Bittner, J. J. Genesis of breast cancer in mice. *Texas Rep. Biol. & Med.*, 10: 160, 1952.
80. Leuchtenberger, C. Cytoplasmic "inclusion bodies" containing desoxyribose nucleic acid (DNA) in cells of human rectal polyps. *Lab. Investigation*, 3: 132, 1954.
81. Leuchtenberger, C., Doolin, P. F. and Kutsakis, A. H. Buffered osmium tetroxide (O_8O_4) fixation for cytological and Feulgen microspectrophotometric studies of human rectal polyps. *J. Biophys. & Biochem. Cytology*, 1: 385, 1955.
82. Albright, F., Burnett, C. H., Smith, P. H. and Parson, W. Pseudo-hypoparathyroidism, an example of "Seabright-bantam syndrome." Report of three cases. *Endocrinol.*, 30: 922, 1942.
83. Stanbury, J. B. and Querido, A. Genetic and environmental factors in cretinism; a classification. *J. Clin. Endocrinol. & Metab.*, 16: 1522, 1956.
84. Gruneberg, H. An Annotated Catalogue of the Mutant Genes of the House Mouse. Medical Research Council Memorandum No. 33. London, 1956. H. M. Stationery Office.
85. Grodsky, G. M. and Carbone, J. V. "Direct" bilirubin production in rat tissue homogenates. *Clin. Research Proc.*, 5: 78, 1957.
86. Calkins, E. Personal communication.
87. Huestis, R. R., Anderson, R. S. and Motulsky, A. G. Hereditary spherocytosis in peromyscus. I. Genetic studies. *J. Hered.*, 47: 225, 1956.

Pathogenetic Problems in Phenylketonuria*

W. Eugene Knox, M.D. *and* David Y.-Y. Hsia, M.D.

Boston, Massachusetts

Only twenty years elapsed between Følling's original description of phenylketonuria [*38*] as an hereditary metabolic abnormality characterized by the excretion of phenylpyruvate and by mental deficiency, and the development of a rational therapy for this disease by Bickel [*13*] and Armstrong and Tyler [*15*]. In the intervening years Jervis [*57*] had identified the metabolic abnormality as an inability to oxidize phenylalanine to tyrosine



and had demonstrated directly that the liver enzyme which normally catalyzed the reaction was inactive in this disease [*60*]. The latter finding, in 1953, closed an important chapter in the study of phenylketonuria. For the first time the three postulates of Garrod's "inborn errors of metabolism" [*42*], the relationship of gene, enzyme and clinical abnormality, were convincingly demonstrated.

Until the present time the factors favoring research in this disease have been (1) a simple and precise diagnostic test in the green color reaction of ferric chloride with the phenylpyruvate of urine; (2) the segregation of the rare patients with this disease (one in 25,000 to 40,000 in the population) into institutions where they made up about 1 per cent of the mental defectives [*61*]; and (3) the conceptual basis of the disorder implicit in the concept of "inborn errors of metaboiism." Henceforth, the problems to be solved will concern the nature of the gene-enzyme relationship and the relationship of the biochemic abnormality to the mental deficiency.

Both of these relationships can be included in a consideration of the pathogenesis from gene to intelligence. The mechanisms of pathogenesis will be discussed in steps of increasing complexity, from genetics and biochemistry to pathologic physiology and clinical signs. The earlier and simpler phenomena can be thought of as causes of the later and more complex events.

It would be rash even to attempt such a codification of a disease process without a solid foundation of facts. The manifold facets of phenylketonuria were authoritatively reviewed in 1954 by Jervis [*61*]. The background of biochemical processes in aromatic amino acid metabolism has been thoroughly covered by Dalgliesh [*32*]. Even so, the main virtue of this provisional outline of the pathogenesis of phenylketonuria may be to throw into relief the gaps awaiting study.

Genetic Analysis. Few human diseases are so uniformly manifested and precisely identifiable as phenylketonuria. Because of this, its incidence can readily be shown to conform to the Mendelian laws of inheritance and a high degree of certainty resides in the statements about its genetic distribution. The mode of inheritance of phenylketonuria has been deduced from the histories of families in which these cases occur in so complete and satisfactory a manner that only the conclusions need be given [*40,54,61,75*].

Phenylketonuria is a recessive condition, transmitted by a single autosomal gene. It occurs in each sex with equal frequency, since the gene is not located on the sex chromosomes. The main features of the disease are the result of one gene, and are not the composite result of several genes. The single responsible gene must be very similar or identical in different families

* From the Cancer Research Institute, New England Deaconess Hospital, and the Departments of Biological Chemistry and Pediatrics, Harvard Medical School, Boston, Massachusetts. Supported by grants from the U. S. Atomic Energy Commission and U. S. Public Health Service.

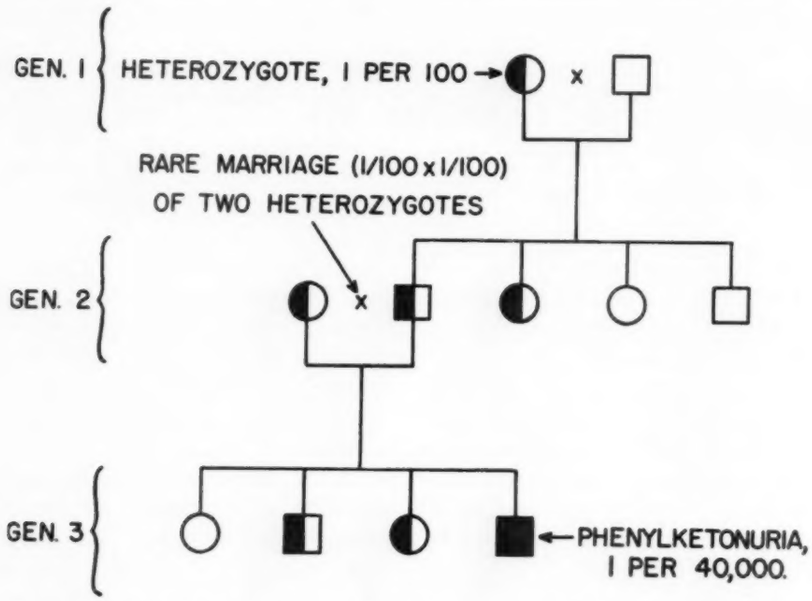


FIG. 1. Idealized inheritance pattern of phenylketonuria. An apparently normal heterozygote (half-black symbol) in Generation 1 will transmit his gene to half his progeny (Generation 2). About $\frac{1}{100}$ of the population are heterozygotes, and the rare marriage between two heterozygotes (less rare in consanguineous marriages) will produce, on the average, one phenylketonuric offspring (full black symbol) in four. Note that no family history of the disease would be given in this case.

because of the uniformity of the disease picture. The condition occurs only in persons who receive two of the abnormal genes, one from each parent. When this happens the disease invariably develops. The absence of an analogous, milder disease in the heterozygote, who carries only one abnormal gene, is well established. The typical inheritance of phenylketonuria (Fig. 1) is therefore from two apparently normal parents (heterozygotes). One in four of their children, on the average, will have phenylketonuria, one will be normal, and two apparently normal (heterozygotes).

The distribution of the gene in an average family has been determined by statistical methods based on the distribution of phenylketonuric patients in known families. The single gene can now also be identified directly by a chemical test which distinguishes the heterozygotes [*49*]. With this method statements can be made about persons instead of populations, but the facts cited will not be altered. Instead of one in four, none or more than four phenylketonuric children may be produced by a given family, since the lottery of genes at each fertilization is an independent event, like the tossing of a coin. Only in the aggregate, including those families with none affected, will the ratio of affected to normal children approach one in four. It can also be seen in Figure 1 that the usual presentation of a rare hereditary disease like phenylketonuria will be a single case appearing without known antecedent in the family. The incidence in a sibship and in a family (the family history) is therefore of no value in excluding heredity as the cause of a rare, recessive disorder.

Considerable interest is attached to the question of whether or not the heterozygotes for phenylketonuria are quite normal. A possible increased susceptibility to involutional psychoses in later life was mentioned by Følling, and supported by Penrose [*77*]. Data on large series of families [*54,75*] show that this is not usual; but few heterozygotes have been definitely identified (parents only) and fewer still have been seen when they could manifest a disease of old age. There are about a hundred unidentified heterozygotes for every heterozygote known through his phenylketonuric offspring. The calculated frequency of heterozygotes in the population ($2\sqrt{\text{incidence}}$) is somewhat more than one per one hundred people, or about the same frequency

as mental illness in general. The presence of any diathesis in such a sizable fraction of the population could be missed in the few known parents and still might account for considerable morbidity. The identification of any diathesis which may result from a single recessive gene will constitute the major clinical contribution made by tests to detect heterozygotes of recessive diseases.

Gene-Protein Relationship. Disparate hereditary characters are often associated with a single gene (pleiotropism). The most satisfactory explanation of this phenomenon is that the characters are different secondary effects of a single gene action. To believe that urinary excretion of phenylpyruvate and intelligence have a common denominator may require an act of faith; but the studies of phenylketonuria provide important support for the lowest common denominator of gene action which has been widely demonstrated in nature: the one-to-one relation of gene action with an enzyme action [*7*]. Although an enzyme is often said to be "missing" or "absent" in a patient with an inborn error of metabolism or in a mutant *Neurospora*, only its activity is missed. It is quite probable that the enzyme molecule is there, but structurally altered in keeping with the mutation of its genic template. The enzyme may be more or less functionless as a result of the change. That, at least, is the situation in respect to the abnormal hemoglobin diseases [*51*], the prototypes of the molecular diseases [*76*].

It is a tenable hypothesis that phenylketonuria also originates in the malfunctioning of a structurally altered enzyme protein, and that all the signs and symptoms can be explained by this hereditary molecular abnormality. There is direct evidence for the primary malfunctioning of one enzyme, and only one, in phenylketonuria. Chemical anomalies in metabolic sequences of compounds other than phenylalanine do occur. Multiple enzymic "blocks" have been invoked to account for these other biochemical disturbances [*18*]. This entails abandoning the unitary hypothesis of gene action [*2,31*], and it should be resisted so long as other explanations could also account for the findings. (See *Pathogenesis.*)

Diminished oxidation of phenylalanine to tyrosine explains the most constant and central biochemical findings in phenylketonuria. Direct evidence of this is the accumulation of phenylalanine [*39,56*], the lack of a transient rise in blood tyrosine after phenylalanine administration [*57*], and the very limited (only 10 per cent of the normal) conversion of labelled phenylalanine to tyrosine [*90*]. The leakage of 10 per cent through the block has been ascribed to a possible alternative pathway but it may well be the measure of the residual activity of the altered enzyme molecule. Autopsy samples of phenylketonuric livers were inactive in oxidizing phenylalanine to tyrosine when collected and measured under conditions in which active extracts were obtained from other human livers [*60*]. Inactivity could result secondarily from the diseased state, so inactivity does not prove an "absent" or defective enzyme. Phenylalanine hydroxylase can be inhibited, for example, by certain α-keto acids [*91*], one of which saturates the phenylketonuric tissues (phenylpyruvate). But there is recent and important support for the view that phenylalanine hydroxylase inactivity is the primary phenomenon in this disease. The phenylalanine tolerance tests [*49*] already mentioned can identify the apparently normal heterozygote. The heterozygotes have none of the secondary chemical or clinical abnormalities seen in the phenylketonuric patient but they do show a definitely diminished capacity to metabolize large doses of phenylalanine. This is indicated by the higher blood levels and lagging disposal of the amino acid, presumably caused by less than normal phenylalanine hydroxylase activity. The presence of some defective enzyme in these bearers of only one defective gene would be predicted on the basis of the one-to-one gene-enzyme relationship, if the enzyme defect were the primary abnormality.

The direct demonstration of a molecular defect in the enzyme remains to be accomplished. The lability of phenylalanine hydroxylase [*91*] has discouraged its study as a protein. Recently it has been recognized that a group of enzymes, including phenylalanine hydroxylase, have many properties in common [*44*]. These enzymes all add molecular oxygen to their substrate, and for activity require a heavy metal, certain reducing agents like DPNH or TPNH, and oxygen. The mechanism of the enzyme reaction is not yet known, but the two enzymes [*73*] needed for the oxidation of phenylalanine apparently include one which acts merely to form the needed DPNH from added DPN. If this is so, there is no longer any basis for the assumption of a two-step oxidation of phenylalanine, one step of which was considered to be absent in

phenylketonuria, and the other to give rise to the abnormal hydroxyphenyl compounds in the urine [*3,18*].

SIGNS AND SYMPTOMS OF PHENYLKETONURIA

The thesis maintained here is that every effort should be made to identify all abnormalities in phenylketonuria, and to explain them either as near or remote results of diminished phenylalanine oxidation, so far as this can be done. The effort is more than an exercise in ingenuity, since identification of even one regular feature not ultimately explicable by the presumed primary enzymic lesion will mean that there is a still more fundamental lesion underlying the enzymic abnormality.

The purpose of this section is merely to list and to evaluate the abnormalities observed in this disease. Numerous associated conditions have been reported in occasional phenylketonuric patients, and some of these have gained a certain acceptance as part of the disease picture, more by repetition than by corroboration. Only those findings are included here which have been made with reasonable consistency in published and in personally observed cases. A more elaborate codification of the abnormalities to be explained in phenylketonuria is sorely needed.

Chemical Substances. The aromatic compounds found in abnormal amounts in phenylketonuria are given in Table 1 as derivatives of their parent amino acids. The major ones are L-phenylalanine, and its -pyruvate, -lactate, -acetate [*61*] and -acetylglutamine derivatives [*72,86,96*]. The body fluids contain a high concentration of phenylalanine (20 to 60 mg. per cent in plasma) but there is less of the remaining compounds, which are more rapidly cleared by the kidneys. Amounts of the order of a gram or more of each of these compounds may be excreted in the urine per day, depending on the body size and the diet. Free phenylacetic acid may be present in a lesser amount, and some may also be conjugated with glucuronic acid [*61*]. The phenylalanine derivatives were found and measured by classic chemical methods. There is no doubt about their presence and no great difficulty in accounting for their formation.

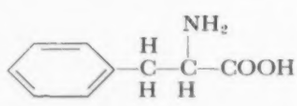
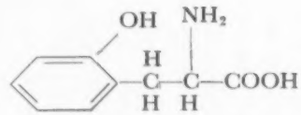
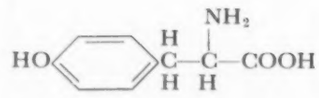
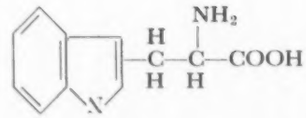
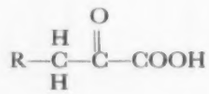
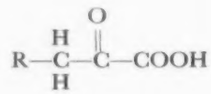
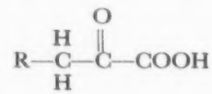
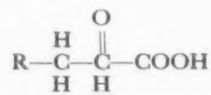
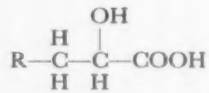
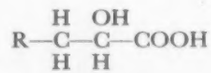
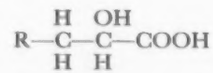
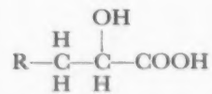
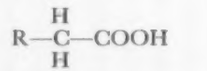
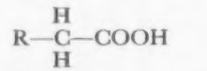
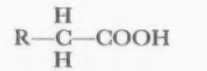
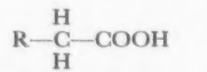
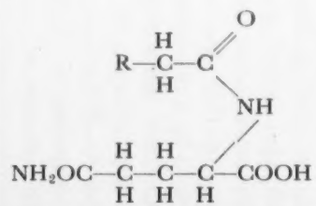
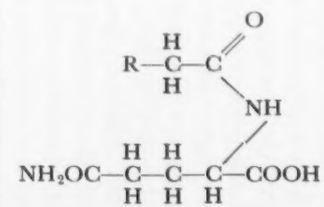
The remaining compounds are excreted in smaller amounts and were detected since the advent of paper chromatography. They probably include substances reported earlier but unidentified [*9,29,34*]. They consist of the same derivatives of three other amino acids as the derivatives of phenylalanine, with some exceptions, and without the excretion of abnormal amounts of the parent amino acids. *o*-Hydroxyphenylacetate [*4,19*] is excreted in an amount of 0.1 to 0.4 gm. per day [*3,4*]. Indolylacetate and indolyllactate [*2,52*] are excreted in amounts of 0.02 to 0.15 gm. per day or less. These three compounds have been chemically isolated and identified, as well as demonstrated by paper chromatography. *p*-Hydroxyphenyllactate and -acetate [*18*], and *p*-hydroxyphenylpyruvate (K. R. Rees, cited in [*12*]), have been detected only chromatographically and no measure of the amounts excreted is available. The qualitative detection in urine of a physiologic compound does not in itself constitute an abnormality. An average of 0.04 gm. per day of *p*-hydroxyphenylpyruvate has been reported in the urine of a great variety of patients, including those with liver disease, blood dyscrasias and miscellaneous infections [*43*]. It is also claimed that *m*-hydroxyphenyl-acetate and -lactate are regularly present in normal urine but are absent from the urine in phenylketonuria [*18*]. Neither of these statements has yet been confirmed.

The organization of Table 1 implies that the missing derivatives of each amino acid can potentially be formed and might also be found in the urine in phenylketonuria. *o*-Hydroxyphenyllactate was not found, however, even after administration of its present compound, *o*-tyrosine [*3*]. Indolylacetylglutamine was identified in the urine of patients with the recently described Hartnup disease (also called H-disease, [*53*]) but the same method did not reveal its presence in the urine in phenylketonuria [*52*]. *o*-Hydroxyphenylacetate apparently cannot be conjugated with glutamine by man [*24*].

Jervis and his group considered the possibility that the incorporation of phenylalanine into body protein might be abnormal in phenylketonuria. Analysis of serum, brain, liver and kidney protein from phenylketonuric patients showed a normal phenylalanine content [*16*]. These results can be accepted despite a report to the contrary [*82*]. It remains possible that the synthesis of a particular protein in a given tissue might be altered and that this would not be detected by an analysis of the proteins of the tissue *en bloc*. Something like this has been detected in an electrophoretic study of plasma proteins in phenylketonuria. Multiple spike anomalies of the β-lipoproteins were found in eight of eighteen

TABLE I

ABNORMAL METABOLITES IN PHENYLKETONURIA

	PRIMARY		SECONDARY	
	Phenylalanine	*o*-Tyrosine	Tyrosine	Tryptophan
				
Derivatives:	Phenyl = R	*o*-Hydroxyphenyl = R	*p*-Hydroxyphenyl = R	Indolyl = R
-pyruvic:				
-lactic:				
-acetic:				
-acetyl glutamine:		-acetylglutamine not formed in man	-acetylglutamine not formed in man	

The compounds within the boxed area have been found in the urine in phenylketonuria. All are derivatives of the parent amino acids indicated at the tops of the columns. The primary metabolites come from phenylalanine and the secondary metabolites from other amino acids, possibly by adapted reactions.

patients examined [*22*]. The anomalies diminished on a phenylalanine-deficient diet. No ultracentrifuge studies were reported. A somewhat similar anomaly was found in some cases of primary systemic amyloidosis [*79*].

Pigmentation. The limitation of the first examples of the disease, found in Norway, to fair-haired, fair-skinned persons with blue eyes was not particularly remarkable. But over 90 per cent of all patients seen in different countries, including the United States, are said to conform to this type [*61*]. The disease may be more frequent in, but is definitely not limited to, people of North European origin. Cases have been described in typical Mediterranean families, [*54,88*], one case in a Negro [*37*], and two cases in one Jewish family [*27*]. All that can be said is that the coloration of affected subjects is lighter than that of the family, and this dilution of pigment has been confirmed spectroscopically [*30*] and is reversed by treatment [*5,13,20,85,97*]. Some patients are relatively well-pigmented, although as a group their coloration tends to resemble the Nordic type.

Dermatologic Abnormality. Observers have often commented on the tendency of these patients, especially in the early years of life, to have some kind of skin disease. One-third of 104 published case reports examined mentioned some abnormality of the skin. This varied from severe eczemoid lesions to mild eruptions or simply to dry, rough or scaly skin. No dermatologic appraisal of these lesion appears to have been made, nor is there any comparison of their frequency with the frequency of similar lesions among other defective children. There is a tendency to have a fine and probably easily damaged skin, as well as a light skin. The abnormality could be no more than an increased sensitivity of the skin to the usual traumas experienced by a defective child, especially since the lesions are most common on wetted and exposed areas.

Epinephrine Tolerance. An increased hypertensive response to intravenous epinephrine injections has been reported to occur in phenylketonuric patients [*25*]. These observations were made in two patients and are at present unconfirmed, even though the technic has been used to evaluate patients with various other kinds of mental disease [*41*]. The finding is sufficiently important for it to be repeated, and if necessary, to be extended to include more precise measurements of epinephrine sensitivity such as its effects on carbohydrate metabolism. Confirmation of a sort was the low plasma epinephrine level found in thirteen phenylketonuric children [*93*], although any mental defect will produce a level lower than that of normal persons.

Central Nervous System Abnormalities. These may be divided for convenience into (1) general neurologic findings, (2) epilepsy and electroencephalographic abnormalities, and (3) the mental defect. It should be stressed at the outset that no neuropathologic findings (or any pathologic findings in other parts of the body) are regularly present which might account for the clinical abnormalities observed. The sole finding which has recurred in several cases, usually in children [*1,61*] but also in two adults [*8*], is localized or diffuse demyelination or retarded myelination. Such lesions are not inconsistent with what might be expected in this kind of metabolic disease, nor with the neurologic findings in some cases. Many other autopsies did not show these abnormalities. The existence of such lesions in at least a definite fraction of these cases remains to be established. But the presence of a functional abnormality is suggested by the decreased removal of oxygen from the blood by the brain in nine phenylketonuric patients compared with that in undifferentiated mental defectives of the same age [*45,46*].

Neurologic Findings. These have been well summarized by Jervis [*61*]. In about two-thirds of the cases slight to marked neurologic signs are present. These usually consist of extra-pyramidal signs with some degree of hyperactivity of the deep reflexes, muscular hypertonicity, ataxia and athetoid movements or tremors. Rarely are pyramidal signs present, although the gross movements in many cases remind one of patients with cerebral palsy. Restlessness expressed in incessant, repetitive and manneristic motion is very common.

Epilepsy. One-fourth to one-third of phenylketonuric patients have or have had seizures, usually of the grand mal type. These episodes tend to decrease in frequency with age. It is probable that petit mal attacks are even more common in early life. The expected electroencephalographic abnormalities are regularly observed in those patients with epilepsy. Minor abnormalities of the electroencephalogram are reported in the majority of patients, including those not subject to fits [*74*]. Kreezer attempted to correlate the several properties of the electro-

encephalogram with mental age in different conditions [*66*], and found what he considered to be a characteristic pattern of abnormalities in phenylketonuric patients [*67*]. The evaluation and interpretation of any abnormalities of the electroencephalogram which might be characteristic of phenylketonuria would greatly aid our understanding of the neurologic disorder. By providing an objective sign, this would undoubtedly help in the development of successful treatment.

Mental Defect. It is clear that the majority of phenylketonuric patients are of low-grade intelligence, except possibly during the first few months of life. All have some degree of mental defect. Rarely, if ever, does a patient show any progressive deterioration as part of the major disease process. It is said that they do not differ psychologically from other subjects with mental defects.

There is direct evidence that none are of normal intelligence. Tests of 10,000 young men in military service by Jervis [*67*] and of 8,220 people in southern Sweden not known to be mentally defective [*68*] did not turn up a single case. To these may be added an unknown number of persons whose urine has been tested with ferric chloride by physicians who would have recognized the abnormality. These surveys of nearly 20,000 normal people provide direct proof that the true incidence of phenylketonuria is close to that calculated on the incidence in the mentally defective population. The calculated incidence may be in error only to the extent that the rare case with borderline intelligence escapes institutionalization.

The majority of patients with phenylketonuria are idiots. Perhaps one-half of them never learn to talk, and one-third never learn to walk. But a definite fraction of them are educable, and some are "borderline mental defectives" (I. Q. 75 to 80). Jervis found the following distribution of I. Q. among a total of 330 patients for whom exact psychometric data were available:

I. Q.	<10	11–20	21–30	31–40	41–50	51–60	61–70	>70
Per cent	37	26	12	10	9	2	1	0.9

It is important to establish that typical phenylketonuria is compatible with relatively high grade (borderline) intellectual development. Cases of this sort have been reported, often with the suggestion that they represented partial chemical blockage, heterozygotes, mild variants of the gene, and so on. These cases have been recently reviewed in connection with careful studies of a thirteen year old girl who had phenylketonuria and who was only one year behind her age group in public school [*50*]. All the typical chemical and clinical parameters of this disease, except for the relatively high I. Q., were confirmed in this patient. The parents and a brother were identified as heterozygotes by phenylalanine tolerance tests. The patient and a sister, a typical institutionalized phenylketonuric with an I. Q. of less than 10, were undoubtedly homozygous for the gene. In three other families with one phenylketonuric member with borderline intelligence there was also a second member who was a severely retarded phenylketonuric subject. In the family studied it was clear that there was no significant difference in the degree of interference with phenylalanine oxidation between the two patients with borderline and low intelligence, and that the gene present in this family was not limited to a mild effect on intelligence. The explanation of relatively exceptional intelligence in phenylketonuric patients, or of the wide spread in I. Q., has been sought only in the amounts of possibly "toxic" metabolites excreted, and without success. Solution of this problem will greatly advance our understanding of the pathogenesis and possibly improve the treatment of phenylketonuria.

PATHOGENESIS

The major abnormal metabolites, phenylalanine and its derivatives, are produced by the overflow of large amounts of phenylalanine through reactions normally carrying only small quantities. The combined leakages are sufficient to re-establish a steady state in the face of the dietary intake only at extremely high blood levels of phenylalanine. At these levels the renal reabsorptive capacity is overwhelmed and a significant amount of phenylalanine is lost in the urine. There is no specific renal impairment [*36*]. More phenylalanine is lost by chemical changes. The nature of these changes establishes a pattern for the degradation of excess amounts of any aromatic amino acid. (Table I.) Phenylpyruvate is first formed, almost certainly in part by transamination [*72*], and possibly also by the action of L-amino acid oxidase [*14*]. Part of the phenylpyruvate is reduced to phenyllactate, a reaction readily catalyzed by DPN lactic dehydrogenase

[*71*]. Another portion is oxidatively decarboxylated to phenylacetate. The particular enzymes which do this to aromatic α-keto acids are not yet known but the reaction is a familiar one in tissues. The conjugation with glutamine of phenylacetate fed to man and higher apes has long been known [*87,96*]; the reaction is probably analogous to that of the enzyme which forms hippuric acid [*80*].

The same series of derivatives, allowing for certain exceptions caused by the chemical specificities of the body, may be expected in the disposal of excessive amounts of any of the aromatic amino acids. A similar pattern of metabolites of tryptophan is found in the newly described "Hartnup disease" [*6*]. Excess tryptophan is excreted in the urine, and although the labile indolylpyruvate and its reduced product, indolyllactate, have not been found, indolylacetate and indolylacetylglutamine are both excreted [*53*]. *p*-Hydroxyphenylpyruvate, its lactate [*69*] and the acetate derivatives [*63*] are excreted when the metabolism of tyrosine is inhibited by scurvy.

The most puzzling biochemical aspects of phenylketonuria are the origins of those metabolites analogous to the ones from phenylalanine, but derived from three other aromatic amino acids: *o*-tyrosine, tyrosine and tryptophan. (Table 1.) The *o*-hydroxyphenylacetate is definitely formed from phenylalanine, as was shown by C^{14} labelling experiments [*92*]. The ortho-hydroxylation cannot be affected by the para-directing phenylalanine hydroxylase, but perhaps by some still undiscovered ortho-hydroxylase [*44*]. Acting on phenylalanine, phenylpyruvate or phenylacetate, this would provide one of the escape routes that carries off the excess phenylalanine. Dalgliesh demonstrated that "unspecific hydroxylation" by an artificial system could give rise to most of the abnormal metabolites found in phenylketonuria [*37*]. It is reasonable to believe that enzyme systems exist in the body to duplicate these results. It has been speculated that the loss of one of two steps in the reaction of the phenylalanine hydroxylase could give rise to abnormally hydroxylated phenyl compounds [*3,19*]. No experimental basis remains for assuming two steps for this reaction but, even so, this theory does not account for the abnormal tryptophan derivatives. The multiple metabolic blocks proposed [*18*] to account for the compounds excreted is a phenomenologic explanation. It is unlikely in view of the normal metabolism of large amounts of tyrosine [*55*] and *o*-tyrosine [*3*] in these patients. More importantly, as already pointed out, its acceptance incurs abandonment of the theory of the unitary action of the gene.

If it is assumed that increased conversion of *o*-tyrosine, tyrosine and tryptophan to their pyruvate derivatives occurs in phenylketonuria, the other abnormal metabolites excreted also could be produced by analogous mechanisms. *o*-Tyrosine is not a known component of the body and must be formed from phenylalanine or phenylpyruvate as already described. The pyruvate derivatives of these three amino acids might then be expected to give the several abnormal compounds so far found, and potentially several others, just as does phenylalanine, and as do tryptophan in Hartnup disease and tyrosine in scurvy.

The reason for the increased formation of the pyruvate derivatives of phenylalanine and *o*-tyrosine, at least, appears to be obvious. There is excess phenylalanine which must be disposed of through whatever reactions are available. However, it is unlikely that the normal metabolic machinery could perform any new reactions, or even the normal subsidiary reactions on the observed scale without a sort of chemical hypertrophy, an adaptive increase in certain enzymes. It is probable that appropriate metabolic adjustments are made over the long period of time that the phenylketonuric subject is stimulated by the high tissue levels of phenylalanine. Ortho hydroxylation of phenylalanine normally is undetectable, but becomes a major reaction in phenylketonuria. An adaptive increase in a transaminase or oxidase may be necessary before significant amounts of phenylpyruvate can be formed. This would account for the lag period during the first weeks or months of life before a phenylketonuric child begins to excrete phenylpyruvate [*47*]. Also, in normal people given phenylalanine tolerance tests [*49*], it was noted that no phenylpyruvate was excreted when the plasma levels of phenylalanine were raised to those at which phenylpyruvate regularly formed in phenylketonuric subjects. Presumably in the phenylketonuric person there is an increased ability to make this compound from phenylalanine. Similar adjustments or adaptive changes in other enzymes may also be expected to occur, since such changes can follow alterations in substrate concentration or hormonal balance, and consequently can be caused by almost any

disturbance of the metabolic state of an organism [*64*].

An adaptive increase in the conversion of phenylalanine to phenylpyruvate, which would at the same time increase the conversion of the other aromatic amino acids to their pyruvate derivatives, could involve either of the projected deaminating reactions. Increased L-amino acid oxidase activity would result in oxidation of more tyrosine and tryptophan [*14*] and *o*-tyrosine [*15*] as well as more phenylalanine to the pyruvate derivatives, since the (rat kidney) enzyme has relatively high activity for all these compounds. Its increased activity in respect to other amino acids would contribute to the cellular imbalance of amino acids to be discussed. An adaptive increase in phenylalanine transamination would not obligatorily entail an increased reaction of the other amino acids, since recent work in this laboratory has shown that separate and specific transaminases exist for each of the amino acids, phenylalanine, tyrosine (see also [*23*]), tryptophan and histidine. Possibly several of these enzymes could be adapted in man by the same stimulus, although phenylalanine did not adapt them in the rat. It is probable, however, that increased amounts of keto acid acceptors (α-ketoglutarate and pyruvate) would be available as a result of the large scale transamination of phenylalanine (by α-keto acids regenerated from glutamate). This could facilitate transaminations in general without significant change in the enzyme concentrations. The reported finding of α-ketoglutarate in the urine of phenylketonuric subjects (Rees, cited in [*12*]), together with the phenylpyruvate present in the blood [*59*], are indications of such an α-keto acid surfeit.

It is clear that the effect of loss of the activity of an enzyme in an organism will involve more than simply omission of one step in an otherwise rigidly maintained metabolic sequence. The metabolism of the whole organism is altered from the normal in other ways besides that represented by the primary defect. This adaptability accounts for the survival of organisms with such fundamental lesions as those of an hereditary disease, but it increases the complexity of the pathogenetic mechanisms. No further effort need be made to analyze the possible physiologic consequences of the enzymic adaptations already mentioned, until evidence as to their true nature and extent is available. But secondary disturbances of another sort must also be mentioned. The amino acids are normally concentrated within cells by more or less specific transport systems. The total amount and the proportion of one to another is of undoubted importance to the cell's normal function. Christensen [*26*] has drawn attention to the importance of the possible derangement of cellular amino acid balance caused by the excess of phenylalanine in the tissues of the phenylketonuric subject. No complete analyses of blood or tissue free amino acids are yet available in this disease, and the extent of the induced disproportion cannot be assessed. But serious harm, of an unexpected sort, could be produced by the displacement by phenylalanine of some other essential amino acid from some particular tissue. Treatment designed to restore biochemic normality by decreasing the phenylalanine level in tissues might defeat itself unless the mixture of amino acids fed also permitted a normal cellular free amino acid pattern to be re-established.

The abnormal β-lipoproteins in phenylketonuric serum could represent one consequence of this cellular amino acid imbalance. Liver dysfunction was suggested as a cause of these anomalies [*22*], although the only clinically recognizable liver dysfunction found generally in phenylketonuria is the specific inability to oxidize phenylalanine. The structure of lipoproteins, with their propensity for binding small molecules, usually lipids, possibly even phenylalanine, might be particularly sensitive to the chemical milieu in the cells where they are made.

The subnormal pigmentation and the sensitivity to epinephrine can be discussed together, since both melanin and epinephrine are products of tyrosine metabolism [*32*]. The cells which produce these substances, the melanophores and cells of the adrenal medulla, are both derived from the neural crest [*48*]. A diminished synthesis of epinephrine, producing an effect like Cannon's Law on denervated organs, would result in a greater response to a given dose. The syntheses of both pigment and epinephrine may therefore be inhibited. Defective differentiation of these tissues is one possibility. This is unlikely, even though phenylalanine (or tyrosine) is required for the normal differentiation of these tissues, and phenyllactate can specifically inhibit the differentiation of the pigment cells [*94,95*]. Even with normal differentiation, an inadequate tyrosine supply might restrict the similar functions of these two related tissues.

An overt deficiency of tyrosine probably does

TABLE II

PROVISIONAL PATHOGENETIC CHAIN OF CAUSATION IN PHENYLKETONURIA

The primary enzymic abnormality at the left "in some way causes" the sequential disturbances in the columns to the right.

			"Toxic" metabolite (?)			
			Phenylacetate odor			
		Overflow of phenylalanine to phenyl- and o-hydroxyphenyl- derivatives	Adapted conversion of tyrosine and tryptophan	Cerebral oxygen uptake reduced		
	Phenylalanine accumulation			Electroencephalographic abnormalities	Neurologic signs	
		Altered amino acid pattern in cells		Skin sensitivity		
Defective phenylalanine hydroxylase	Tyrosine deficiency		Abnormal β-lipoproteins	Light coloration	Epilepsy	Mental deficiency
		Abnormal tissues from neural crest	Inhibited pigmentation	Sensitivity to epinephrine	Skin lesions	
			Inhibited epinephrine synthesis			

not occur in phenylketonuria, even though tyrosine is an essential amino acid for these patients. Tyrosine is an essential amino acid for most cells, except those like liver cells which can form it from phenylalanine [*35*]. There is no significant dwarfing or subnormal growth of phenylketonuric subjects to suggest an amino acid deficiency if the somewhat smaller physical size of mentally defective subjects in general is used as the norm. Nevertheless, a relative deficiency may exist. Normal diets contain no great excess of tyrosine. Very much less than the normal amount of tyrosine was found in the urine of one phenylketonuric patient [*36*], and a lower than normal plasma tyrosine level in another [*13*]. Any abnormal excretion of *p*-hydroxyphenylpyruvate and its derivatives represents lost tyrosine. Any competition with the structurally related phenylalanine present in large excess could diminish a marginal tyrosine metabolism to critical levels. Evidence of a relative tyrosine deficiency, or of an unfavorable phenylalanine-tyrosine competition, is afforded by the darkening of new grown hair when either the tyrosine intake was increased (by massive doses for three months) [*85*] or the phenylalanine intake was lowered [*5,13,20,97*]. An analogy for this effect is the competitive inhibition by phenylalanine of mushroom tyrosinase activity [*33*]. This system is commonly used as a model of the still obscure pigment-forming system in man. If the epinephrine sensitivity in phenylketonuria can be confirmed, it will be of interest to see if conditions restoring pigmentation also decrease sensitivity to epinephrine.

A direct inquiry into the origin of the central nervous system abnormalities is still impossible because of the multiplication of the hypothetic secondary and tertiary effects of what is actually known to be abnormal at a lower level of complexity. (Table II.) Certain limitations can be delineated, however. These concern the probable time and nature of the injury, and the possible toxicity of certain metabolites.

The tissues showing abnormalities in phenylketonuria, the central nervous system, skin and possibly adrenal medulla, are embryologically derived from ectoderm. No serious disturbance of the differentiation of these tissues in fetal life occurs, at least no detectable morphologic indi-

cation of this is found, and the probability of any intrauterine damage is small. Fetal abnormalities or stillbirths do not occur in excess in phenylketonuric families or the effect of this would have appeared in the genetic statistics. The affected children are uniformly considered well for the first few months of life. It is unlikely that a phenylketonuric fetus in its heterozygous mother would accumulate any excess phenylalanine, when the normal supply is only adequate for the rapid rate of protein synthesis taking place. The report that a phenylketonuric woman bore two normal children [*61*] provides additional evidence that exposure to excessive phenylalanine levels during gestation does not harm the fetus.

The involvement of the three tissues of similar origin must therefore be attributed to the similarity of their chemical functions or susceptibilities, and not to their differentiation. The abnormalities probably develop after birth. If this is so, the clinical signs of phenylketonuria are acquired as a result of the built-in biochemical environment to which the three tissues are exposed during infancy.*

The simplest abnormality of the internal environment to be measured against the mental defect, and the only one considered to date, is a hypothetic "toxic" compound among the abnormal metabolites in this disease. It has been assumed that a parallelism will be found between the amount of this compound and the I. Q. in a series of patients, although corrections for body size and food intake have not been used in such studies. In six patients between five and sixty-two years of age receiving the same diet *ad libitum*, the hourly output of phenylpyruvate was found to be in proportion to body surface area, and to bear no relationship to the I. Q. of the patients [*65*]. Two small children with relatively high I. Q.'s excreted relatively little phenylpyruvate [*28,40*], but these amounts were probably the usual ones for their size.

In general, no correlation has been found between I. Q. and the blood levels of phenylalanine [*4,17*] or of phenylpyruvate [*59*], or the urinary excretion of phenylalanine, phenyllactate and phenylpyruvate [*58*] or *o*-hydroxyphenylacetate [*4*]. The suggestion that D-phenylalanine is formed by racemization and does the damage has been disproved [*78*]. The amounts of the indole compounds and of phenylacetylglutamine excreted remain to be measured in relation to body size and I. Q.

Frequent references are made to the toxicity of phenylacetate, which was noted many years ago in the course of experiments to find a limit to the ability of the body to make glutamine [*84*]. Massive doses, several times the amount formed in phenylketonuric subjects, were taken repeatedly by volunteer subjects, with some discomfort but no truly alarming symptoms [*83*]. Bickel, in the course of toxicologic studies of several of the metabolites, administered extremely large doses of phenylacetate to a phenylketonuric child without significant effect on her behavior [*12*]. Definite effects were observed, consisting of slight ataxia and deterioration of behavior, when sufficient extra phenylalanine (or phenylpyruvate) was given to increase the blood level of phenylalanine [*10,12*]. Only one clinical manifestation was clarified by the experiments with phenylacetate. The "musty" or "mousey" odor of the urine in phenylketonuria was greatly increased by the administration of extra phenylacetate, which has itself a sweetish, musty odor.

It is not necessary for a single "toxic" compound, in a pharmacologic sense, to be found to account for the abnormalities of the central nervous system in phenylketonuria. Too many chemical disturbances are known which would also have some effects. The increased tissue concentration of some metabolites and decreased amounts of others, and the amplification of these abnormalities by adaptive changes of enzymes, altered amino acid uptake and possible deficiency of regulators like epinephrine, could so displace the chemical milieu from its normal homeostatic balance that normal function of cells could not be maintained. The decreased cerebral arteriovenous oxygen difference is one sort of indication of malfunction, and the abnormal electroencephalographic activity and clinical behavior are indications of another sort of the same malfunction. Despite its complex origin, however, the effect might be compared to that of anesthesia or stupefaction by a simple pharmacologic agent. The effects of deprivation of learning during a life-long anesthesia would not be reversed as promptly as the anesthesia itself! It is necessary to separate

* This view was confirmed by a recent study of a newborn patient. The serum phenylalanine level was normal at birth (Armstrong, M. D. and Binkley, E. L., Jr. *Proc. Soc. Exper. Biol. & Med.*, 93: 418, 1956). In addition, the formation of the various metabolites lagged behind the accumulation of phenylalanine until the necessary enzyme adaptations occurred.

the effects on neurologic signs, electroencephalographic changes and stupidity from what we recognize by empiric standards as intelligence. Intelligence standards are derived from normal persons who develop without pause in the absence of high tissue levels of phenylalanine. When the phenylalanine level in phenylketonuria is restored to normal and the pharmacologic effects are reversed, it will be necessary to face the problem of determining intelligence in new kinds of creatures, either modern Galateas or monsters.

TREATMENT

This is neither the time nor place for a definitive evaluation of the most recent therapy of phenylketonuria. Only short term accounts of approximately seventeen patients treated with phenylalanine-deficient diets have been published [*5,11,13,20,47,97*]. At least several times this number of patients are currently being treated. For the present purpose the various forms of therapy which have been tried will be considered only as "therapeutic trials," important for what information they contribute about the nature of the disease.

The literature abounds in references to uniformly unsuccessful treatments with various mineral, vitamin or hormonal supplements. The rationale behind these attempts may have been based on misunderstanding of the true nature of the disease, or on the wishful thought that available ancillary components of enzyme systems might replace a whole enzyme. The results support the view that only a gene can make an enzyme. An intriguing possibility, attributed to Linus Pauling in the popular press, would entail the introduction into an artery of a catalyst-lined tube to take over the specific oxidation of phenylalanine for the organism. No catalyst with the required properties is now known.

Tyrosine supplementation of the diet of "tyrosineless" phenylketonuric patients has been given adequate clinical trial [*12,29,70,81,85*]. Despite the possibility of a relative tyrosine deficiency in these patients, as already discussed, tyrosine supplementation by itself has had no effect except to increase hair pigmentation when given in large doses. It is noteworthy that this regimen disclosed no evidence of a secondary block [*18*] in tyrosine metabolism. Prolonged treatment with glutamic acid was unavailing [*98*].

All current treatment involves the production of a partial phenylalanine deficiency to eliminate the accumulation of phenylalanine in these patients. Up to the present the overriding consideration has been to determine whether or not it was possible to maintain a patient with adequate nutrition for a sufficiently long period on a low phenylalanine diet so that the effects of this treatment could be determined. The early and limited use of diets supplying nitrogen in the form of a costly mixture of pure amino acids (by Armstrong and Tyler [*5*] and Dent, cited in [*5*]) was supplemented by the use of casein hydrolysates treated to remove the phenylalanine. This type preparation, which could be made on the necessary large scale, was suggested by Woolf and was first used late in 1951 by Bickel and his associates [*13*] as the basis of a diet on which a two year old girl was maintained for six weeks. Two commercial concerns* undertook to supply this diet for several long-term experiments. Various refinements of the diet were made to increase acceptance and to insure an adequate intake of essential amino acids, accessory food factors and calories. It now appears that, with care, at least the majority of children can be maintained in positive nitrogen balance and show a satisfactory rate of growth on such diets, while at the same time the blood phenylalanine levels are significantly lowered. The earliest patients treated have been maintained in this way for over three years [*11,89*].

The phenylalanine-deficient diet has almost uniformly produced results which support the concept that most, if not all, of the disease manifestations are secondary to the accumulation of phenylalanine. It remains only to ascertain, through longer trials, closer biochemical control and adequate testing of the patients treated, whether or not complete relief of all signs and symptoms can be achieved. Editorial comments on this development have been guarded in tone [*21,62*], largely because what we recognize as normal intelligence has not yet been demonstrated in a treated phenylketonuric patient. The objective evidences of improvement leave little doubt that the disease can be substantially alleviated. Possibly development of the disease can be prevented by this treatment, since the youngest patients treated are not demonstrably abnormal.

The biochemical abnormalities characteristic of

* Allen and Hanbury, Ltd. in England and Merck & Co. in the United States.

phenylketonuria were completely eliminated by the low phenylalanine intake. This occurred in about a month or less, depending upon the phenylalanine intake, the adequacy of the diet and the growth potential retained. The urinary excretion of phenylpyruvate was a poor index of control since it disappeared while the plasma phenylalanine was still elevated [*5*]. When the plasma phenylalanine was reduced to near-normal levels there was no further urinary excretion of phenylalanine, phenylpyruvate [*13*], phenyllactate, phenylacetylglutamine [*65*] and *o*-hydroxyphenylacetate [*4,18*]. The peculiar odor of the urine vanished [*5,13*]. The *p*-hydroxyphenyl compounds, which may not be present in abnormal amounts, remained unchanged. Some indolylacetic acid persisted in a reduced amount, but this was probably derived from the DL-tryptophan present in the diet [*65*]. The abnormal β-lipoproteins found by plasma electrophoresis substantially disappeared [*22*].

The clinical signs in phenylketonuric patients also improved after several months of treatment during which a negative urine phenylpyruvate test was maintained. The skin of those patients with dermatologic lesions cleared [*5,13,20*] and in most patients new-grown hair was darker [*5,13,20,97*]. Convincing objective evidence of the clinical value of the treatment was the decrease in number, or the abolition of, the epileptic seizures and the disappearance of electroencephalographic abnormalities in at least eight patients who were originally affected in this way [*5,12,20,47,97*]. The precipitation of severe epileptiform seizures during the first month of treatment while the phenylalanine was being depleted (in three cases referred to in the literature [*5,12,97*] and a fourth with fatal result that has been communicated to us) may give warning of the risk of a critical complication in readapting the phenylketonuric patient to a new way of life.

There is near unanimity that changes in the neurologic behavior were produced by the diet. Increased attentiveness and composure are the attributes most commonly reported. There are few detailed comparisons of the neurologic findings before and after treatment, since these apparently fade in importance in relation to the distinct upsurge of general development seen while treatment continues. The Griffiths mental development scale for testing infants from birth to two years was used to good advantage in describing the changes [*97*], since it represents a method of numerically grading the clinical observations on five distinct aspects of development. Significant improvement occurred in all aspects, and similar results are to be found in the other reports. Mental age and I. Q. have generally increased slightly, less than would be expected from the clinical improvement, and possibly only because of the improved testability of the patients. But only the extent and not the fact of the effectiveness of the diet hinges upon the I. Q. changes.

It should be noted that all aspects of the disease except the mental deficiency have been reversed in relatively short periods. In the published cases the patients range in age at the start of therapy from eight weeks [*47*] to eight years [*12*], with possibly better results in the younger patients. No age distinction was apparent, however, in the degree of biochemical or objective clinical improvements. The suggestion has been made that treatment should be started in the first few months of life "before irreversible brain damage has occurred" [*11*]. While early diagnosis should be encouraged, it is not yet clear that any irreversible changes do occur or that the younger children do respond better. Because chronologic age is a factor in all intelligence measurements, older children are put at a numerical disadvantage when they begin to develop [*97*].

In evaluating and comparing the results of treatment, more emphasis must be placed on the degree of biochemical control achieved, since this remains the touchstone of effectiveness. The goal should be the maintenance of biochemical normality, including the correction of any amino acid imbalance or relative tyrosine deficiency which may prove to occur, and the avoidance of nutritional stress. It is a tribute to modern nutritional science that people can survive on this unnatural diet, and it would not be unexpected if residual chemical abnormalities should persist or be created. However, the results already obtained with a certain degree of chemical control should encourage work to eliminate any remaining chemical abnormalities in phenylketonuric patients.

REFERENCES

1. ALVORD, E. C., STEVENSON, L., VOGEL, F. S. and ENGEL, R. L. Neuropathological findings in phenylpyruvic oligophrenia (phenylketonuria). *J. Neuropath. & Exper. Neurol.*, 9: 298, 1950.
2. ARMSTRONG, M. D. and ROBINSON, K. S. On the

excretion of indole derivatives in phenylketonuria. *Arch. Biochem. & Biophys.*, 52: 287, 1954.

3. Armstrong, M. D. and Shaw, K. N. F. The metabolism of *o*-tyrosine. *J. Biol. Chem.*, 213: 805, 1955.
4. Armstrong, M. D., Shaw, K. N. F. and Robinson, K. S. The excretion of *o*-hydroxyphenylacetic acid in phenylketonuria. *J. Biol. Chem.*, 213: 797, 1955.
5. Armstrong, M. D. and Tyler, F. H. Studies on phenylketonuria. I. Restricted phenylalanine intake in phenylketonuria. *J. Clin. Investigation*, 34: 565, 1955.
6. Baron, D. N., Dent, C. E., Harris, H., Hart, E. W. and Jepson, J. B. New pellagra-like disease with aminoaciduria. *Lancet*, 2: 421, 1956.
7. Beadle, G. W. Biochemical genetics. *Chem. Rev.*, 37: 15, 1945.
8. Benda, C. E. Developmental Disorders of Mentation and Cerebral Palsies. New York, 1952. Grune & Stratton.
9. Berry, J. P. and Woolf, L. I. Estimation of phenylpyruvic acid. *Nature*, 169: 202, 1952.
10. Bickel, H. Effects of phenylalanine-free and phenylalanine-poor diet in phenylpyruvic oligophrenia. *Exper. Med. & Surg.*, 12: 114, 1954.
11. Bickel, H. Diagnosis and therapy of galactosemia and phenylketonuria. *Monatschr. Kinderh.*, 103: 81, 1955.
12. Bickel, H., Boscott, R. J. and Gerrard, J. In: Biochemistry of the Developing Nervous System, p. 417. Edited by Waelsch, H., New York, 1955. Academic Press.
13. Bickel, H., Gerrard, J. and Hickmans, E. M. The influence of phenylalanine intake on the chemistry and behavior of a phenylketonuric child. *Acta Paediat.*, 43: 64, 1954.
14. Blanchard, M., Green, D. E., Nocito, V. and Ratner, S. L-amino acid oxidase of animal tissue. *J. Biol. Chem.*, 155: 421, 1944.
15. Blaschko, H. Decarboxylation of *o*-hydroxyphenylalanine. *Biochem. J.*, 44: 268, 1949.
16. Block, R. J., Jervis, G. A., Bolling, D. and Webb, M. The amino acid content of tissue proteins of normal and phenylpyruvic oliogophrenic individuals. *J. Biol. Chem.*, 134: 567, 1940.
17. Borek, E., Brecher, A., Jervis, G. A. and Waelsch, H. Oligophrenia phenylpyruvica. Constancy of the metabolic error. *Proc. Soc. Exper. Biol. & Med.*, 75: 86, 1950.
18. Boscott, R. J. and Bickel, H. Detection of some new abnormal metabolites in the urine of phenylketonuria. *Scandinav. J. Clin. & Lab. Investigation*, 5: 380, 1953.
19. Boscott, R. J. and Kirman, B. H. Urinary phenolic acid excretion in phenylketonuria. *Biochem. J.*, 60: iv, 1955.
20. Braude, H. Phenylketonuria, a case report in a European child treated with a diet low in phenylalanine. *South African M. J.*, 30: 83, 1956.
21. Editorial. *Brit. M. J.*, 1: 93, 1955.
22. Brown, D. M., Armstrong, M. D. and Smith, E. D. Studies on phenylketonuria. IV. Serum proteins in phenylketonuria. *Proc. Soc. Exper. Biol. & Med.*, 89: 367, 1955.
23. Canellakis, Z. N. and Cohen, P. P. Kinetic and substrate specificity study of tyrosine-α-ketoglutaric acid transaminase. *J. Biol. Chem.*, 222: 63, 1956.
24. Cerecedo, L. R. and Sherwin, C. P. Comparative metabolism of certain aromatic acids. V. Fate of some ring substitution products of phenylacetic acid in the organisms of the dog, rabbit and man. *J. Biol. Chem.*, 58: 215, 1923–1924.
25. Cawte, J. E. Phenylketonuria. *M. J. Australia*, 2: 15, 1954.
26. Christensen, H. N. Metabolism of amino acids and proteins. *Ann. Rev. Biochem.*, 22: 235, 1953.
27. Cohen, P. and Kozinn, P. Phenylpyruvic oligophrenia in a Jewish child. *J. Pediat.*, 34: 76, 1949.
28. Cowie, V. An atypical case of phenylketonuria. *Lancet*, 1: 272, 1951.
29. Cowie, V. Phenylpyruvic oligophrenia. *J. Ment. Sc.*, 97: 505, 1951.
30. Cowie, V. and Penrose, L. S. Dilution of hair colour in phenylketonuria. *Ann. Eugenics*, 15: 297, 1951.
31. Dalgliesh, C. E. Unspecific hydroxylation reactions in the metabolism of aromatic amino acids. *Biochem. J.*, 58: xlv, 1954.
32. Dalgliesh, C. E. Metabolism of the aromatic amino acids. *Adv. Prot. Chem.*, 10: 31, 1955.
33. Dancis, J. and Balis, M. E. A possible mechanism for disturbance in tyrosine metabolism in phenylpyruvic oligophrenia. *Pediatrics*, 15: 63, 1955.
34. Dobriner, K., Rhoads, C. P. and Lieberman, S. Spectroscopy as applied to the study of phenylpyruvic oligophrenia. *Research Publ., Nerv. & Ment. Dis.*, 22: 158, 1943.
35. Eagle, H. The specific amino acid requirements of a human carcinoma cell (strain HeLa) in tissue culture. *J. Exper. Med.*, 102: 37, 1955.
36. Evered, D. F. The excretion of amino acids by the human. *Biochem. J.*, 62: 416, 1956.
37. Ferriera-Fernandes, J. Oligofrenia fenilpiruvica em melanoderma. *Brazil med.*, 64: 225, 1950.
38. Følling, A. Ueber Ausscheidung von Phenylbrenztraubensäure in den Harn als Stoffwechselanomalie in Verbindung mit Imbezillität. *Ztschr. f. physiol. Chem.*, 227: 169, 1934.
39. Følling, A. and Closs, K. Ueber das Vorkommen von l-Phenylalanin in Harn und Blut bei Imbecillitas phenylpyrouvica. *Ztschr. f. physiol. Chem.*, 254: 115, 1938.
40. Følling, A., Mohr, O. L. and Ruud, L. Oligophrenia Phenylpyruvica. A Recessive Syndrome in Man. Oslo, 1945. Dybwad.
41. Funkenstein, D. H., Greenblatt, M. and Solomon, H. C. Autonomic changes paralleling psychologic changes in mentally ill patients. *J. Nerv. & Ment. Dis.*, 114: 1, 1951.
42. Garrod, A. E. Inborn Errors of Metabolism, 2nd ed. London, 1923. H. Froude, Hodder & Stoughton.
43. Gros, H. and Kirnberger, E. J. Spontanausscheidung von *p*-Oxyphenylbrenztraubensäure im Harn. *Klin. Wchnschr.*, 32: 115, 1954.
44. Hayaishi, O. et al. Symposium on enzymatic activation of molecular oxygen. *Abst. Am. Chem. Soc.*, p. 53, 1956.
45. Himwich, H. E. and Fazekas, J. F. Cerebral metabolism in mongolian idiocy and phenylpyruvic oligophrenia. *Arch. Neurol. & Psychiat.*, 44: 1213, 1940.

46. Himwich, H. E. and Fazekas, J. F. Cerebral arteriovenous oxygen difference. II. Mental deficiency. *Arch. Neurol. & Psychiat.*, 51: 73, 1944.
47. Horner, F. A. and Streamer, C. W. Effect of a phenylalanine-restricted diet on patients with phenylketonuria. *J. A. M. A.*, 161: 1628, 1956.
48. Hörstadius, S. The Neural Crest. London, 1950. Oxford University Press.
49. Hsia, D. Y., Driscoll, K., Troll, W. and Knox, W. E. Detection by phenylalanine tolerance tests of heterozygous carriers of phenylketonuria. *Nature*, 178: 1239, 1956.
50. Hsia, D. Y., Knox, W. E. and Paine, R. S. A case of phenylketonuria with borderline intelligence. *J. Dis. Child.* (In press.)
51. Itano, H. A., Bergren, W. R. and Sturgeon, P. The abnormal human hemoglobins. *Medicine*, 35: 121, 1956.
52. Jepson, J. B. Paper chromatography of urinary indoles. *Lancet*, 2: 1009, 1955.
53. Jepson, J. B. Indolylacteyl-glutamine and other indole metabolites in Hartnup disease. *Biochem. J.*, 64: 14, 1956.
54. Jervis, G. A. The genetics of phenylpyruvic oligophrenia. *J. Ment. Sc.*, 85: 719, 1939.
55. Jervis, G. A. Metabolic investigations on a case of phenylpyruvic oligophrenia. *J. Biol. Chem.*, 126: 305, 1938.
56. Jervis, G. A., Block, R. J., Bolling, D. and Kanze, L. Phenylalanine content of blood and spinal fluid in phenylpyruvic oligophrenia. *J. Biol. Chem.*, 134: 105, 1940.
57. Jervis, G. A. Studies of phenylpyruvic oligophrenia. The position of the metabolic error. *J. Biol. Chem.*, 169: 651, 1947.
58. Jervis, G. A. Excretion of phenylalanine and derivatives in phenylpyruvic oligophrenia. *Proc. Soc. Exper. Biol. & Med.*, 75: 83, 1950.
59. Jervis, G. A. Studies on phenylpyruvic oligophrenia. Phenylpyruvic acid content of blood. *Proc. Soc. Exper. Biol. & Med.*, 81: 715, 1953.
60. Jervis, G. A. Phenylpyruvic oligophrenia. Deficiency of phenylalanine oxidizing system. *Proc. Soc. Exper. Biol. & Med.*, 82: 514, 1953.
61. Jervis, G. A. Phenylpyruvic oligophrenia (phenylketonuria). *Research Publ. Nerv. & Ment. Dis.*, 33: 259, 1954.
62. Queries and minor notes. *J. A. M. A.*, 160: 88, 1956.
63. Kirberger, E. and Bucher, T. Ausscheidung von *p*-Oxyphenylessigsäure nach Belastung mit *p*-Oxyphenylbrenztraubensäure beim Kaninchen. *Biochim. et Biophys. Acta*, 8: 294, 1952.
64. Knox, W. E., Auerbach, V. H. and Lin, E. C. C. Enzymatic and metabolic adaptations in animals. *Physiol. Rev.*, 36: 164, 1956.
65. Knox, W. E. and Edwards, S. W. Unpublished.
66. Kreezer, G. L. Abstract. *Proc. Am. A. Ment. Defic.*, 42: 1304, 1937.
67. Kreezer, G. L. In: Intelligence: Its Nature and Nurture, p. 130. Edited by Whipple, G. M. National Society for Study of Education, Yearbook 39. Bloomington, 1939. Public School Publishing Co.
68. Larson, C. A. An estimate of the frequency of phenylketonuria in South Sweden. *Folia Hered. et Path.*, 4: 40, 1954.
69. Levine, S. Z., Marples, E. and Gordon, H. H. A defect in the metabolism of tyrosine and phenylalanine in premature infants. *J. Clin. Investigation*, 20: 199, 1941.
70. Mautner, H. and Quinn, K. V. Phenylpyruvic oligophrenia. *Ann. Pediat.*, 172: 1, 1949.
71. Meister, A., Reduction of α, γ-diketo and α-keto acids catalyzed by muscle preparations and by crystalline lactic dehydrogenase. *J. Biol. Chem.*, 184: 117, 1950.
72. Meister, A., Udenfriend, S. and Bessman, S. P. Diminished phenylketonuria in phenylpyruvic oligophrenia after administration of L-glutamine, L-glutamate or L-asparagine. *J. Clin. Investigation*, 35: 619, 1956.
73. Mitoma, C. and Leeper, L. C. Enzymatic conversion of phenylalanine to tyrosine. *Federation Proc.*, 13: 266, 1954.
74. Monnier, M. and Horneffer, C. Etude electroencephalographique d'un cas d'oligophrénie phénylpyruvique. *Schweiz. Arch. f. Neurol. u. Psychiat.*, 66: 414, 1950.
75. Munro, T. A. Phenylketonuria. Data on forty-seven British families. *Ann. Eugenics*, 14: 60, 1947.
76. Pauling, L. Abnormality of hemoglobin molecules in hereditary hemolytic anemias. *Harvey Lect.*, 49: 216, 1954.
77. Penrose, L. S. Inheritance of phenylpyruvic amentia. *Lancet*, 2: 192, 1935.
78. Prescot, B. A., Borek, E., Brecher, A. and Waelsch, H. Microbiological determination of L- and D-phenylalanine and of phenyllactic acid. *J. Biol. Chem.*, 181: 273, 1949.
79. Rukavina, J. G., Block, W. D., Jackson, C. E., Falls, H. F., Carey, J. H. and Curtis, A. C. Primary systemic amyloidosis. *Medicine*, 35: 239, 1956.
80. Schachter, D. and Taggart, J. V. Glycine *N*-acylase: purification and properties. *J. Biol. Chem.*, 208: 263, 1954.
81. Schöenberg, H., Schmidt, D. and Wönchaus, I. Phenylpyruvic oligophrenia. Observations on twelve cases. *Ztschr. f. Kinderh.*, 77: 363, 1955. Abstract in: *J. A. M. A.*, 160: 914, 1956.
82. Schrappe, O. Zur pathologische Physiologie der Phenylbrenztraubensäure. *Schwachsinn. Nervenart*, 23: 175, 1952.
83. Sherwin, C. P. and Kennard, K. S. Toxicity of phenylacetic acid. *J. Biol. Chem.*, 40: 259, 1919.
84. Sherwin, C P., Wolf, M. and Wolf, W. The maximum production of glutamine by the human body as measured by the output of phenylacetylglutamine. *J. Biol. Chem.*, 37: 113, 1919.
85. Snyderman, S. E., Norton, P. and Holt, L. E., Jr. Effect of tyrosine administration in phenylketonuria. *Federation Proc.*, 14: 450, 1955.
86. Stein, W. H., Paladini, A. C., Hirs, C. H. and Moore, S. Phenylacetylglutamine as a constituent of normal human urine. *J. Am. Chem. Soc.*, 76: 2848, 1954.
87. Thierfelder, H. and Sherwin, C. P. Phenylacetylglutamin, ein Stoffwechsel Produkt des menschlichen Korpers nach Eingabe von Phenylessigsäure. *Berichte*, 47: 2630, 1914.

88. Turpin, R., Dagand, H., Duchene, H. and Delbarre, F. Présentation clinique d'un malade atteint d'oligophrénie phénylpyruvique. *Ann. M. Psychol.*, 105: 65, 1947.
89. Tyler, F. H., Bosma, J. F. and Armstrong, M. D. Abstract. *Am. Soc. Clin. Invest.*, 34: 917, 1955.
90. Udenfriend, S. The hydroxylation of phenylalanine and antipyrine in phenylpyruvic oligophrenia. *J. Biol. Chem.*, 203: 961, 1953.
91. Undenfriend, S. and Cooper, J. R. The enzymatic conversion of phenylalanine to tyrosine. *J. Biol. Chem.*, 194: 503, 1952.
92. Undenfriend, S. and Mitoma, C. In: Symposium on Amino Acid Metabolism, p. 876. Edited by McElroy, W. D. and Glass, B. Baltimore, 1955.
93. Weil-Malherbe, H. The level of adrenaline in human plasma and its relation to mental activity. *J. Ment. Sc.*, 101: 733, 1955.
94. Wilde, C. E., Jr. The role of phenylalanine in the differentiation of neural crest cells. *Ann. New York Acad. Sc.*, 60: 1015, 1955.
95. Wilde, C. E., Jr. The relationship between phenylalanine metabolism and the differentiation of the neural crest cells. *J. Morphol.*, 97: 313, 1955.
96. Woolf, L. I. Excretion of conjugated phenylacetic acid in phenylketonuria. *Biochem. J.*, 49: 9, 1951.
97. Woolf, L. I., Griffiths, R. and Moncrieff, A. Treatment of phenylketonuria with a diet low in phenylalanine. *Brit. J. Med.*, 1: 57, 1955.
98. Woolf, L. I. and Vulliamy, D. G. Phenylketonuria with a study of the effect upon it of glutamic acid. *Arch. Dis. Child.*, 26: 487, 1951.

Galactosemia*

A. Holzel, M.D., D.C.H., G. M. Komrower, M.B., M.R.C.P., *and* V. Schwarz, PH.D., B.SC.

Manchester, England

Galactosemia is an inborn error of metabolism characterized by a specific inability to metabolize galactose in the normal manner, a partial block occurring in the conversion of galactose to glucose. This may result in the development of a high level of galactose in the blood, widespread tissue damage and interference with growth [*1–11,12,14,15,21–23,31,34,35*]. The condition is uncommon although, with growing awareness of the disorder, more cases are being discovered each year.

Inheritance. Many workers have reported the frequency with which the condition is found in certain families [*11–15*] but the mode of transmission of the gene has been a matter for speculation. As the galactose tolerance test is abnormal in all cases of galactosemia, the authors used it in all the near relatives in four families in whom there were affected children [*16*]. The subjects were all carefully examined to exclude other causes of galactose intolerance (such as liver disease). A galactose index [*17*] of more than 160 was considered abnormal.

In every family one parent had an abnormally high galactose index and in one instance both parents were found to have a marked impairment of tolerance for galactose. In one family in which there was a marriage between first cousins two children died in infancy and two living children had abnormal galactose indexes. (Fig. 1.) The deaths, both from the clinical description and from autopsy reports, appear to have been due to galactosemia. Recently Berger [*18*] reported on a patient whose parents were first cousins but in whom no estimations of galactose tolerance were carried out.

The investigation revealed a high incidence of abnormal galactose tolerance without any clinical evidence of galactosemia among the members of these families. It was suggested that this state might be inherited as an heterozygous character and the subjects in whom the active overt form develops may be homozygous for the same gene. There might, alternatively, be other factors that accentuate the condition.

The weakness of the argument lies in the fact that we were unable to show abnormal tolerance in both parents in all cases but it is probable that the galactose tolerance test is not a sufficiently

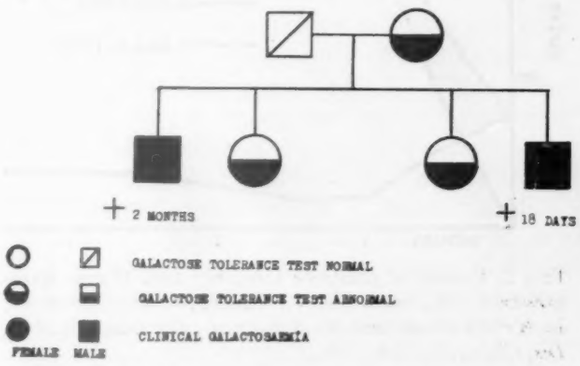


Fig. 1. Family in which parents were first cousins.

sensitive method of investigation. It will be shown later that the enzyme deficiency can be demonstrated in the red blood cells of subjects with galactosemia [*19*] but that the enzyme is present in apparently normal amounts in the erythrocytes of the parents and near relatives. The test, therefore, may not be suitable for genetic investigations. Galactose is largely metabolized in the liver and estimation of the enzyme activity of the liver cells of relatives would probably afford more information. It would not be justifiable to carry out liver biopsies in healthy subjects and the galactose tolerance test remains, therefore, the best method of investigation of heredity at the present time.

Clinical Picture. The disorder may present in one of three ways. In severe cases signs may appear during the first two weeks of life. Vomiting is usually the first indication of any disturbance and this is followed by jaundice and loss of weight. The liver enlarges steadily and may descend below the level of the umbilicus, ascites may develop and the spleen may often enlarge.

* From the Departments of Child Health and Pathology, University of Manchester.

The infant is reluctant to feed and is lethargic; there is marked hypotonia with failure to thrive and the stools may be loose and frequent. Cataracts develop in more than half the cases and may appear as early as the third week.

In a second group of cases the development of symptoms and signs may be slow and, as a result, the infant is not brought forward for examination for several months. Difficulty with feeding, occasional vomiting and retarded development are the presenting symptoms and on examination hepatomegaly is found and often cataracts. The infant is not acutely ill.

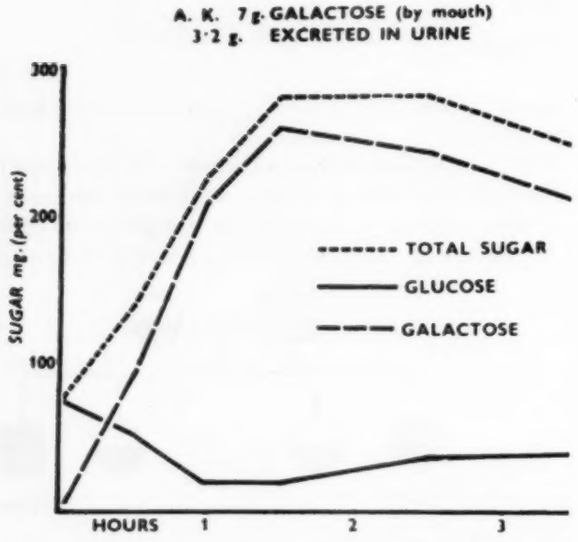


FIG. 2. Curves of galactose tolerance test. (From KOMROWER, G. M., SCHWARZ, V., HOLZEL, A. and GOLDBERG, L. A clinical and biological study of galactosaemia. *Arch. Dis. Child.*, 31: 254, 1956.)

Finally, there are the mild cases which are usually found by chance when a case of galactosemia has led to examination of the other members of the family. These subjects have never liked milk and frequently will give a history of early weaning; they are much healthier and happier when milk is omitted from their diet. They have an abnormal galactose tolerance without any other clinical evidence of liver damage.

Galactosuria and proteinuria are found in cases falling into the first two clinical groups, the galactose being readily demonstrated in the urine by paper chromatography [*7,20,32*]. These signs, however, may not be present in children diagnosed after the age of two years, when the normal diet contains appreciably smaller quantities of milk. The diagnosis is confirmed by the presence of an elevated level of galactose in the blood and by the grossly abnormal galactose tolerance test, which shows high levels of galactose for several hours; in addition, between 40 per cent and 50 per cent of the ingested galactose can be recovered from the urine. (Fig. 2.)

Depression of the blood glucose levels, which has been reported by several authors [*14,21,23–25*], was noted in this investigation and on one occasion symptoms of pallor, sighing respirations and sweating were observed in relation to a hypoglucosemia (18 mg./100 ml.). For this reason it is suggested that the galactose tolerance test should be carried out only if it is difficult to establish a diagnosis without its assistance. The test will be essential, however, for the third clinical group as there is neither galactosuria nor proteinuria but only abnormal galactose tolerance in these cases.

Proteinuria is mentioned in all the previously reported cases. In our experience the amount excreted is substantial, rising to more than 1 gm. per day. Electrophoresis of this protein has shown fractions corresponding to the normal range of serum proteins. Aminoaciduria was first described in 1952 [*26*] and several workers have confirmed this observation [*20,24,27,28*]. The galactosuria, proteinuria and aminoaciduria all disappear when lactose or galactose is removed from the diet.

In untreated infants there is evidence of some disturbance of liver function: a rise of serum bilirubin and serum alkaline phosphatase and a prolongation of the prothrombin time. The figures in no measure compare with the abnormality of galactose tolerance and usually return to normal when the milk or lactose-containing feeds are discontinued, although in cases in which treatment is appreciably delayed irreversible liver damage is sustained, with the ultimate development of a portal cirrhosis.

Hyperchloremic acidosis has been noted by several authors in some untreated cases (*vide infra*); this condition also is corrected by adjustment of the diet. Finally the few serum potassium figures recorded are all on the low side (3.25 to 4.0 mEq./L.) both in treated and untreated children. In one of our cases the potassium level dropped from 3.5 mEq./L. to 2.8 mEq./L. during ten days of galactose feeding.

Treatment. The essentials of treatment are the *rigid* exclusion of all milk, lactose or galactose-containing substances from the diet and correction of any dehydration or acidosis. The very

sick infant will certainly require intravenous or subcutaneous fluids at first, then clear fluids orally and finally the lactose-free diet. Intravenous Hartmann's or Butler's solution mixed with equal parts of a 10 per cent dextrose solution are suitable preparations as they would help to correct the hypoglucosemia, the acidosis and the lower serum potassium: either of these mixtures could then be given orally for a short time.

Most of the special milks prepared for these children do, in fact, include small quantities of lactose; and certain soya milk preparations, although free from lactose, contain some polygalactose and are therefore not suitable. For these reasons a lactose-free feed was introduced by Holzel et al. [*29*]. The feed is based upon a milk-free diet devised by Moll and Stransky and consists of egg, sugar, margarine and rice flour. This is a high carbohydrate feed and may cause some gastrointestinal disturbance in very young infants. It is therefore important to commence with dilute feeds in order to prevent any vomiting or diarrhea and to increase the strength and caloric value very slowly. As a result the initial weight gains are minimal although the general condition of the child improves very quickly and the symptoms and signs regress. Abdec® (0.5 cc. daily) has been given to supply the necessary vitamins and also orange juice or rose-hip syrup; these fruit drinks will augment the potassium intake. It should be remembered that vitamin C and other tablets are often made up with lactose and care must be taken when their use is indicated. In addition, either calcium chloride or calcium gluconate (0.75 gm. daily) should be given twice daily to supplement the low calcium content of the diet. It is advisable to proceed to mixed feeding as soon as the infant has become stabilized on the egg/cereal mixture.

This regimen should also be employed for infants who do not present until they are several months old, as there is no evidence to suggest that they can tolerate lactose in their diet without suffering harm.

This *rigid* exclusion of lactose from the diet should be maintained for three years when a small amount of milk or butter can be allowed in the cooking. We have not permitted cheese in the diet until the fourth year and we do not propose to allow the consumption of any milk drinks or milk foods (for example, ice cream) while the children remain under our care.

The clinically normal persons with abnormal galactose tolerance tests can be given milk in small quantities without apparent harm but these subjects, even when adult, feel healthier when milk is removed from their diets.

Prognosis. In all the discussions on galactosemia the prognosis has been considered to be gloomy, first, in respect to early death [*13,15,31*] and secondly because of certain complications such as cirrhosis of the liver, cataract and mental deficiency [*13,30,31*].

In acute cases early diagnosis and adequate treatment will lessen the mortality rate and reduce the chance of irreversible lenticular and liver damage. It is common experience to see cataracts disappear and liver function return to normal following the introduction of a lactose-free diet.

The possibility of a genetic origin of the mental retardation, independent of the metabolic disorder, has been suggested [*30*]. There is no evidence, as yet, to support the conception of an inherited defect of mental development and we believe the view expressed by Townsend et al. "that mental retardation is a salient complication of the disorder" may not be justified. We have personal experience with three children now aged six years, five years and eight months, and three years, respectively, in whom the diagnosis was made early and who show normal mental development. Persistent hypoglycemia has also been mentioned as a possible cause of mental retardation. This suggestion was first made by Mason and Turner [*21*] but the serial blood glucose estimations [*23,25*] made in patients while on a milk diet do not show a marked hypoglycemia; also galactosemia differs in its clinical picture from hyperinsulinism and glycogen storage disease, disorders characterized by hypoglycemia.

It should be emphasized here that most of the feeds, including nutramigen,® which have been given to many of the subjects described contained small amounts of lactose. This, over a long period of time, may have had a cumulative and therefore a deleterious effect upon cerebral development; in addition, in many cases there had been repeated defections from the diet. Maintenance of a lactose-free diet for at least three years has been emphasized and it is believed that this has an important bearing on the prevention of mental retardation.

The possibility of intrauterine damage has been brought forward by Lockhart and Roboz [*32*] who suggested that in certain cases persistent galactosemia developed in the mother during the

last weeks of pregnancy and as a result an appreciable amount of the galactose crossed the placenta into the infant's circulation. In this way they tried to explain the early onset of severe symptoms in some of the children. In the case they described, galactosuria was detected in

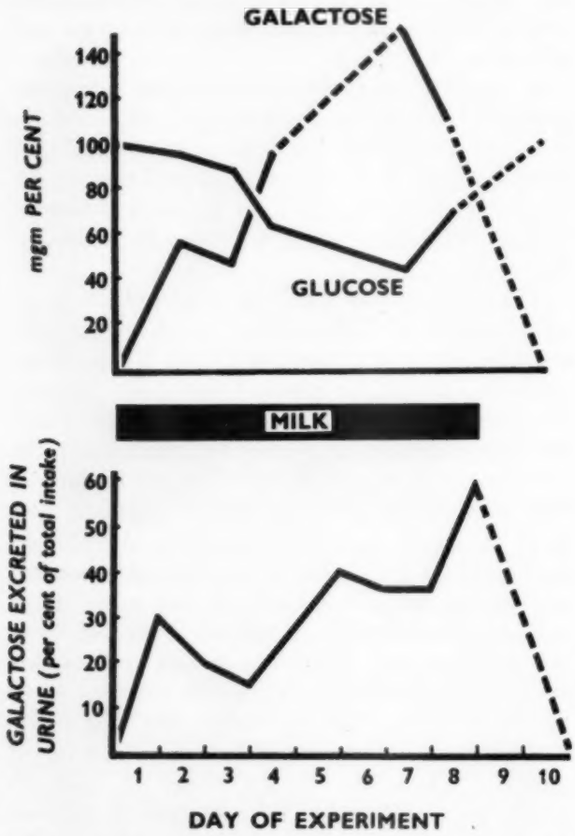


FIG. 3. Blood and urine levels of galactose during a milk feeding observation.

the infant after fifty-two hours; lactose water had, however, been offered from the fourteenth hour and milk introduced at twenty-eight hours. Thus it is almost certain that the galactosuria in this case derived from the ingested lactose. One cannot, however, dismiss their suggestion as no studies have been made of the amniotic fluid of mothers who subsequently have given birth to galactosemic infants; neither have any determinations been made of galactose or galactose phosphate in red cells of the cord blood. (*Vide infra.*) Studies of this nature should be considered as there are reports of a smaller number of infants who died in spite of early diagnosis and the removal of milk from the diet. In some cases it is apparent that small amounts of lactose were left in the diet, in others there may have been a severe degree of acidosis and hypokalemia which was not recognized; but there is a small group left for which there is no satisfactory answer as yet.

Biochemical Investigations. As the result of a detailed investigation of two cases of galacto-

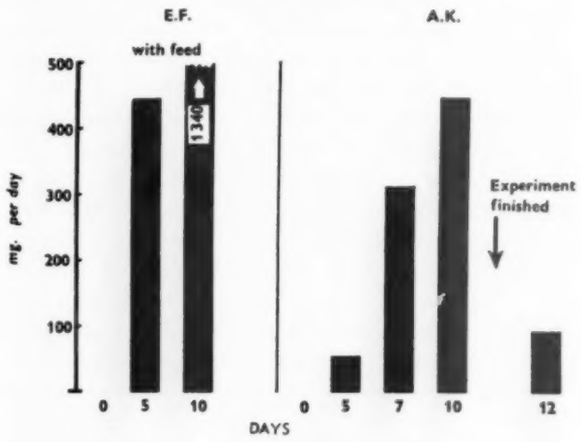


FIG. 4. Proteinuria during feeding observations with galactose (E. F.) and milk (A. K.). (From KOMROWER, G. M., SCHWARZ, V., HOLZEL, A. and GOLDBERG, L. A clinical and biological study of galactosaemia. *Arch. Dis. Child.*, 31: 254, 1956.)

saemia (patients now aged five years and eight months and three years and two months) it was possible to amplify some of the clinical observations mentioned earlier.

Galactose was not observed either in the blood or the urine when the children were on galactose-free diets but an appreciable quantity of blood galactose was detected after twenty-four hours of milk feeding and the level continued to rise throughout the eight days of the observation. Once the milk feeding was stopped, the blood levels dropped rapidly and were zero twenty-four hours after the milk diet had ceased. (Fig. 3.)

The galactosuria increased steadily until the end of the milk or galactose feeding, whereafter there was a rapid fall in the amount of sugar in the urine. In one case no galactose was found in the urine during the second day following the test period.

Proteinuria appeared after forty-eight hours of milk or galactose feeding and increased steadily until the end of the regimen. The proteinuria diminished rapidly following the cessation of milk feeding and disappeared after five or six days. (Fig. 4.)

The *aminoaciduria* came and went in a similar

manner; there was a sudden increase on the fifth or sixth day in the number of amino acids present, with a steady rise of the urine amino-nitrogen level as the feeding continued. The aminoaciduria declined quickly once the milk feeding was stopped and returned to normal after seven to ten days. (Fig. 5.)

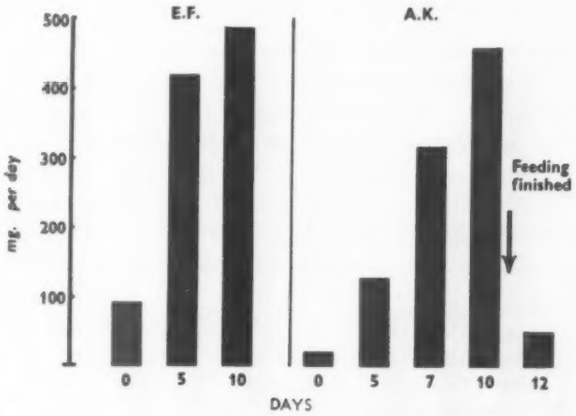


FIG. 5. Urine aminonitrogen levels during feeding observations with galactose (E. F.) and milk (A. K.). (From KOMROWER, G. M., SCHWARZ, V., HOLZEL, A. and GOLDBERG, L. A clinical and biological study of galactosaemia. *Arch. Dis. Child.*, 31: 254, 1956.)

The aminoacid chromatogram showed a relatively large increase in the aminoacids serine, glycine, alanine, threonine, glutamine and valine, with a lesser increase of lysine, cystine, tyrosine and glutamic acid. (Fig. 6.)

The renal origin of this aminoaciduria was first suggested by Komrower [*33*], as the plasma α-aminonitrogen levels were not raised during feeding experiments. This suggestion was substantiated by Cusworth, Dent and Flynn [*24*] who carried out α-aminonitrogen clearances and showed a marked rise in clearance while simultaneous urea clearances remained normal. They reported a slow rise of plasma α-aminonitrogen, although the level never exceeded normal limits. This too was the experience of Hsia and his colleagues [*28*].

An acidosis was noted among the results of routine investigations but its presence was first commented upon by Arthurton and Meade [*34*] who suggested that it might be of renal origin. Berger [*18*] described an hyperchloremic acidosis associated with nephrolithiasis in an infant suffering from galactosemia and suggested that it was similar to the "syndrome of Lightwood-Albright."

An acidosis was first noted in the elder child investigated by the authors when she was twelve months of age and a similar state was observed in the younger child when she was three months old. The blood bicarbonate level returned to normal in each case when a milk-free diet was resumed. (Table I.) It was not possible to reproduce the acidosis in either child during subsequent milk feeding trials hence its exact nature could not be established. There was, however, some suggestion from the results that the acidosis could well be of renal origin as there was an increase of serum chloride, a diminished urine titratable acidity and ammonia content with an increased urine bicarbonate. (Table II.) Their urine became acid whether or not milk was included in the diet. (Fig. 7.)

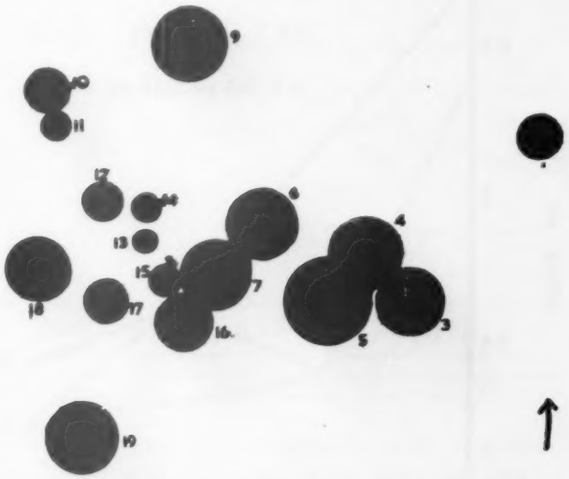


FIG. 6. Urine aminoacid chromatogram in an untreated case of galactosaemia. (The urine has been oxidized to convert cystine and methionine to cysteic acid and methionine sulphone respectively.) Identification of the spots: 1. Cysteic acid. 3. Glutamic acid. 4. Serine. 5. Glycine. 6. Threonine. 7. Alanine. 9. Tyrosine. 10. Phenylalanine. 11. Leucine. 12. Valine. 13. Alpha-amino-n-butyric acid. 14. Methionine sulphone. 15. Histidine. 16. Citrulline. 17. Beta-amino-isobutyric acid. 18. Methylhistidine. 19. Lysine. The amino acid spots are drawn in the form of circles, the relative sizes of the circles giving some indication of the intensity of ninhydrin color and hence of the quantity of each amino acid. The urine has been applied at the right hand bottom corner. Phenol has been run as first solvent from right to left followed by collidine in an upward direction. (From KOMROWER, G. M., SCHWARZ, V., HOLZEL, A. and GOLDBERG, L. A clinical and biological study of galactosaemia. *Arch. Dis. Child.*, 31: 254, 1956.)

The three reversible phenomena just described were the result of temporary renal disturbance. What is the agent responsible for this upset? Many authors [*9,13,23,24,28,35,37*] have suggested that galactose itself is toxic and

that its accumulation in the tissues would interfere with their normal function. It is difficult to verify this in the human subject, as it has been impossible to produce high blood levels of galactose, comparable with those in galactosemia, by feeding the sugar to normal adults or near normal infants. (Personal observations.) On the other hand, galactose feeding does produce cataracts in young rats [*36,38*] and fits in young chicks [*39,40*]. The fits, however, have been shown to be independent of the minor degree of hypoglycemia observed in galactose-fed birds [*41*].

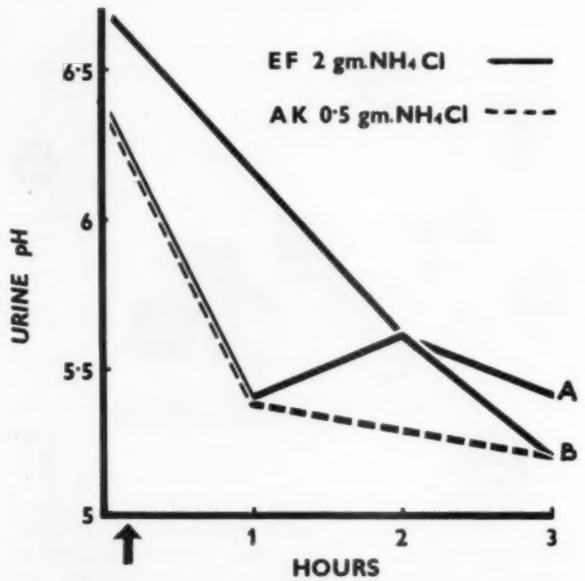


FIG. 7. Acidification of the urine: E. F. (solid line); A, on galactose; B, off galactose; A. K. (broken line), on milk.

Emphasis has been placed earlier upon the rapid appearance of galactose in the blood and urine when milk feeding was commenced and the equally speedy disappearance of the sugar from these fluids when the feeding ceased. It was noted, too, that there was some considerable delay in the appearance and disappearance of the proteinuria and aminoaciduria.

These urinary findings have been variously explained on the basis of (1) competition for reabsorption in the kidney tubule between galactose and amino acids [*26,33*], and (2) the toxic effect of galactose on the tubule cells [*24,28*]. High concentrations of urinary galactose have been noted within twenty-four hours of the start of milk feeding and one would expect any competition between galactose and the amino acids to be manifest immediately. Yet there is no significant increase in the excretion of aminonitrogen until the fifth or sixth day. In like manner the galactosuria disappears twenty-four hours after the cessation of feeding but the increased aminoaciduria persists for many days.

A similar criticism can be advanced against the toxic action of galactose in view of the likelihood of early equilibration between blood and tissue galactose [*42*]. It therefore seemed neces-

TABLE I

PLASMA CO_2 COMBINING POWER ON MILK-FREE AND MILK OR GALACTOSE DIETS

Diet	Date of Test	Plasma CO_2 Combining Power (mEq./L.)	Urine pH
Case E. F.			
Galactose 20 gm. daily			
After 10 days	June 27	11.6	....
After 11 days	June 28	12.9	6.2
Milk- and galactose-free diet for 1 month	August 1	21.0	6.6
Case A. K.			
Milk- and galactose-free diet for 6 weeks	March 10	28	6.42
Milk diet for 8 days	April 6	11.5	7.00
Milk- and galactose-free diet for 20 days	April 27	22.3	5.63

From KOMROWER, G. M., SCHWARZ, V., HOLZEL, A. and GOLDBERG, L. A clinical and biological study of galactosaemia. *Arch. Dis. Child.*, 31: 254, 1956.

TABLE II

SERUM AND URINE BIOCHEMICAL FINDINGS IN A GALACTOSE FEEDING OBSERVATION

	No Galactose (10/11/54)	5 Days Galactose (10/18/54)	10 Days Galactose (10/22/54)
Serum Cl (mEq./L.)	104	.....	108
CO_2-combining power (mEq./L.)	24	.....	22.3
Urine ammonia (mEq./24 hr.)	23	19	11.8
Titratable acidity (Folin) (mEq./24 hr.)	235	164	150
$NaHCO_3$ (mEq./24 hr.)	2	2.47	7.4
Volume (ml.) per day	290	400	445

From KOMROWER, G. M., SCHWARZ, V., HOLZEL, A. and GOLDBERG, L. A clinical and biological study of galactosaemia. *Arch. Dis. Child.*, 31: 254, 1956.

sary to seek a further explanation of this disturbance of cell metabolism.

At least three distinct steps, each catalyzed by a separate enzyme, are required to enable galactose to enter the main stream of carbohydrate metabolism:

$$\text{Galactose} + \text{ATP} \rightarrow \text{Gal-1-P} + \text{ADP} \qquad \text{(Reaction A)}$$

This first reaction is catalyzed by the enzyme galactokinase [*43*]. The galactose-1-phosphate (Gal-1-P) so formed is converted into glucose-1-phosphate (G-1-P) by an enzymic mechanism involving uridine diphosphate glucose (UDPG) as co-enzyme, uridine diphosphate galactose (UDPGal) being an intermediate [*44*].

$$\text{Gal-1-P} + \text{UDPG} \rightleftharpoons \text{G-1-P} + \text{UDPGal} \qquad \text{(Reaction B)}$$

$$\text{UDPGal} \rightleftharpoons \text{UDPG} \qquad \text{(Reaction C)}$$

Reaction C was shown to be catalyzed by the enzyme galactowaldenase [*45*].* More recently Kalckar and his colleagues have identified the enzyme catalyzing reaction B and named it phospho-galactose-uridyl-transferase (P-Gal-transferase) [*19*]. The G-1-P formed from Gal-1-P in reaction B is converted either into glycogen by means of phosphorylase or into G-6-P by means of phospho-glucomutase.

Since most of the ingested galactose is normally metabolized in the liver, the main biochemical lesion must lie in that organ. There is evidence, however, that most, if not all, normal tissues can effect the galactose-glucose transformation to some degree, although this ability may only be of qualitative significance. While liver would be the most obvious tissue to use for the study of the biochemical defect, it is unsuitable on practical grounds and as the abnormal gene responsible for galactosemia is present in every cell of the fully developed organism, other more accessible tissues could lend themselves to close biochemical study.

The presence of the hereditary deficiency in the erythrocytes was first demonstrated in 1955 [*46*]. It was shown that galactose is phosphorylated normally in the galactosemic red cells and that Gal-1-P accumulates, thus indicating the subsequent conversion into G-1-P as the site of the metabolic block. This observation was later confirmed and extended by Kalckar and co-workers [*19*] who established that galactosemic erythrocytes contain little or no P-galactose-uridyl transferase, while the other enzymes concerned with galactose metabolism are present in normal amounts [*47*]. Recently these workers were able to confirm that deficiency of the same enzyme accounts for the impaired function of the galactosemic liver [*48*]. It seems to be established, therefore, that the gene or genes responsible for galactosemia control the synthesis of P-galactose-uridyl-transferase in the cells.

TABLE III
RESPIRATION AND PHOSPHATES OF GALACTOSEMIC ERYTHROCYTES BEFORE AND AFTER ADDING GALACTOSE

	Oxygen Uptake in Presence of Methylene Blue (μl. O_2/hr./10^{10} cells)	Erythrocyte Phosphate Fractions (mg./100 ml. cells)		
		Gal-1-P	Inorg. P	Ester P
Before adding galactose	119.4 ± 4.0	<2	6.96	44.3
After adding galactose	94.4 ± 1.9	17	2.13	36.8

From SCHWARZ, V. A biochemical study of galactosaemia and of the toxicity of galactose. Thesis presented for PH.D. Manchester, 1956.

However, the congenital deficiency of this enzyme does not, in itself, account for the galactosemic syndrome, since the signs and symptoms of the disease become apparent only when galactose is ingested in the diet. These signs, that is the disturbance of liver, kidney, brain and lens function, must result from the "toxic" effect of galactose, or a metabolite of galactose, on cellular metabolism.

The respiration of erythrocytes (in the presence of methylene blue) taken from galactosemic infants before and after a period of galactose feeding was compared. It was found [*49*] that after exposure of the cells to the circulating galactose their oxygen uptake was 20 to 30 per cent lower than before such exposure. Concomitant with this reduction in respiratory activity was a marked fall in the intracellular inorganic P and ester P, the latter in spite of the accumulation of considerable amounts of Gal-1-P. (Table III.)

Similar observations were made when galacto-

* Recently named UDPGal-4-epimerase. (KALCKAR, H. M., MAXWELL, E. S. Some considerations concerning the nature of the enzymic galactose-glucose conversion. *Biochem. et Biophys. Acta*, 22: p. 588, 1956.)

semic erythrocytes were incubated with galactose *in vitro*. On the other hand, when normal erythrocytes were incubated with galactose their respiration was either not affected or actually increased, while their ester P rose.

It can be inferred from these observations that galactose itself is not toxic and that the toxic principle arises only in galactosemic cells as a result of their peculiar enzyme deficiency. Thus it may well be the accumulation of Gal-1-P, or a disturbance of the normal cellular milieu arising therefrom, which is responsible for the partial inhibition of cell metabolism.

It seems likely that a parallel accumulation of Gal-1-P may take place in all or most tissues, and that a partial inhibition of carbohydrate metabolism of these tissues, with consequent disturbance of their function, may ensue. This would account for the observed dysfunction of the kidney, liver, brain and lens, and would, moreover, offer a reasonable explanation for the delay in the appearance and disappearance of proteinuria and aminoaciduria. These aberrations would not become manifest until a critical concentration of the "inhibitor" had been built up, and would not disappear until the inhibitor had been removed.

Although some of the problems associated with this disorder have been clarified during the past few years there are several important questions still to be answered. We have indicated that further information is required before the mode of inheritance can be finally confirmed and it has been suggested that the ultimate prognosis will be given only when carefully treated subjects have been followed up for several years.

The different clinical patterns of the disease that have been described have suggested that the metabolic block is not complete and estimations of galactose intake and output have indicated that some galactose must be metabolized in the individual concerned. Recently Anderson et al. [*52*] have shown that the failure to metabolize galactose in galactosemia is not complete and that the galactosemic subject retains about 2 to 3 per cent of the capacity of a normal person to metabolize the hexose.

It is possible that the enzyme deficiency may vary slightly from one affected subject to another. Further, there may be limited capacity to metabolize galactose by an alternative, although closely related, pathway [*48*]. In addition, the variation between subjects in the rate of organ maturation may determine to what extent a particular organ is affected and, finally, the difference in the amounts of lactose consumed in infants would have a bearing on the severity of the symptoms.

It is now known that the exclusion of galactose from the diet will produce a reversal and, often, complete clearance of all symptoms, with restoration of normal health. Will this removal of galactose give rise to another disease process? Platt [*50*], in another context, has suggested that infants fed on cow's milk (containing smaller amounts of lactose than human milk) may have insufficient galactose available for the synthesis of cerebrosides. This might well render them more prone to have demyelinating conditions of the central nervous system develop later in life.

It is not possible to refute this argument (there appears to be a correlation between the lactose content of the milk and the galactose content of the cerebrosides of different species [*51*]) but it would seem likely that the galactose required for the synthesis of cerebrosides and, indeed, of many other tissue components, is made *in situ* from glucose. The galactose would be incorporated into these compounds through the intermediation of UDPGal and galactowaldenase alone is necessary for this synthesis from glucose.

The question as to whether or not dietary galactose plays a quantitatively significant part in the synthesis of galactolipids is one which cannot yet be answered. It raises the wider problem of the full significance of lactose in the nourishment of the young mammal.

REFERENCES

1. Du Shane, J. W. and Hartman, E. E. Galactosemia in infancy. *Pediatrics*, 7: 679, 1951.
2. Edmonds, A. M., Hennigar, G. R. and Crooks, R. Galactosemia, report of a case with autopsy. *Pediatrics*, 10: 40, 1952.
3. Goldbloom, A. and Brickman, H. F. Galactemia. *J. Pediat.*, 28: 674, 1946.
4. Goldstein, E. O. and Ennis, J. M. Galactosemia. *J. Pediat.*, 33: 147, 1948.
5. Goppert, F. Galaktosurie nach Milchzuckergabe bei angeborenen familiaren chronischen Leberleiden. *Berl. klin. Wchnschr.*, 54: 473, 1917.
6. Greenman, L. and Rathbun, J. C. Galactose studies in an infant with idiopathic galactose intolerance. *Pediatrics*, 2: 666, 1948.
7. Jeune, M., Charrat, A. and Loaec, Y. Un cas de galactosémie du nourisson. *Pédiatrie*, 9: 813, 1954.
8. Maris, L. and Valcke, J. La galactosémie; aperçu général et rélation d'un cas personnel. *Arch. franç. pédiat.*, 10: 36, 1953.
9. Mellinkoff, S., Roth, B. and McLaggan, J. Galactosemia with hepatic damage. *J. Pediat.*, 27: 338, 1945.

10. Reiter, C. and Lasky, M. A. Galactose cataractogenesis. *Am. J. Ophth.*, 35: 69, 1952.
11. Bell, L. S., Blair, W. C., Lindsay, S. and Watson, S. J. Galactose diabetes (galactosemia). *J. Pediat.*, 36: 427, 1950.
12. Donnell, G. N. and Lunn, S. H. Galactosemia; report of four cases. *Pediatrics*, 7: 503, 1951.
13. Townsend, E. H., Mason, H. H. and Strong, P. S. Galactosemia and its relation to Laennec's cirrhosis. *Pediatrics*, 7: 760, 1951.
14. Hudson, F. P., Ireland, J. T., Ockenden, B. G. and White-Jones, R. H. Diagnosis and treatment of galactosaemia. *Brit. M. J.*, 1: 242, 1954.
15. Cox, P. J. N. and Pugh, R. J. P. Galactosaemia. *Brit. M. J.*, 2: 613, 1954.
16. Holzel, A. and Komrower, G. M. A study of the genetics of galactosaemia. *Arch. Dis. Child.*, 30: 155, 1955.
17. Maclagan, N. F. Galactose tolerance as a test of liver function. *Quart. J. Med.*, 9: 151, 1940.
18. Berger, H. La pathogénèse de la galactosémie. *J. Genet. Hum.*, 4: 7, 1955.
19. Kalckar, H. M., Anderson, E. P. and Isselbacher, K. J. Galactosemia, a congenital defect in a nucleotide transferase. *Biochem. Biophys. Acta*, 20: 262, 1956.
20. Bickel, H. and Hickmans, E. M. Paper chromatographic investigations on the urine of patients with galactosaemia (appendix). *Arch. Dis. Child.*, 27: 348, 1952.
21. Mason, H. H. and Turner, M. E. Chronic galactemia. *Am. J. Dis. Child.*, 50: 359, 1935.
22. Norman, F. A. and Fashena, G. L. Chronic hypergalactosemia. *Am. J. Dis. Child.*, 66: 531, 1943.
23. Bruck, E. and Rapaport, S. Galactosemia in an infant with cataracts. *Am. J. Dis. Child.*, 70: 267, 1945.
24. Cusworth, D. C., Dent, C. E. and Flynn, F. V. The aminoaciduria in galactosaemia. *Arch. Dis. Child.*, 30: 150, 1955.
25. Komrower, G. M., Schwarz, V., Holzel, A. and Goldberg, L. A clinical and biological study of galactosaemia. *Arch. Dis. Child.*, 31: 254, 1956.
26. Holzel, A., Komrower, G. M. and Wilson, V. K. Aminoaciduria in galactosaemia. *Brit. M. J.*, 1: 194, 1952.
27. Darling, S. and Mortensen, O. Aminoaciduria in galactosaemia. *Acta paediat.*, 43: 337, 1954.
28. Hsia, D. V., Hsia, H., Green, S., Kay, M. and Gellis, S. S. Aminoaciduria in galactosaemia. *Am. J. Dis. Child.*, 88: 458, 1954.
29. Holzel, A., Komrower, G. M. and Schwarz, V. Low-lactose milks for galactosaemia. *Lancet*, 2: 92, 1955.
30. Clay, P. R. and Potter, C. T. A case of galactosaemia with special reference to mental development. *Arch. Dis. Child.*, 30: 147, 1955.
31. Mortensen, O. and Sondergaard, G. Galactosaemia (galactose disease). *Acta pædiat.*, 43: 467, 1954.
32. Lockhart, J. D. and Roboz, E. Case of galactosemia identified in a four year old infant by paper chromatograph record. *Pediatrics*, 13: 211, 1954.
33. Komrower, G. M. L'aminoacidurie dans la galactosémie. *Arch. franç. pédiat.*, 10: 185, 1953.
34. Arthurton, M. W. and Meade, B. W. Congenital galactosaemia. *Brit. M. J.*, 2: 618, 1954.
35. Bray, P. T., Isaac, R. J. and Watkins, A. G. Galactosaemia. *Arch. Dis. Child.*, 27: 341, 1952.
36. Mitchell, H. S. and Dodge, W. M. Cataracts in rats fed on high lactose rations. *J. Nutrition*, 9: 37, 1935.
37. Johns, D. Galactosemia, an unusual cause of neonatal jaundice *Am. J. Dis. Child.*, 85: 575, 1953.
38. Mitchell, H. S. Cataracts in rats fed on galactose. *Proc. Soc. Exper. Biol. Med.*, 32: 971, 1935.
39. Dain, H. Galactose poisoning in chicks. *Proc. Soc. Exper. Biol.*, 55: 57, 1944.
40. Lecoq, R., Chancard, P. and Mazone, H. Changes in the neuromuscular excitability in acute disturbance of the alimentary equilibrium by carbohydrate. *Compt. rend. Acad. d. Sc.*, 216: 211, 1943.
41. Schwarz, V. A biochemical study of galactosaemia and of the toxicity of galactose. Thesis presented for PH.D. Manchester, 1956.
42. Levine, R., Goldstein, M. S., Huddleston, B. and Klein, S. P. Action of insulin on the permeability of cells to free hexose as studied by its effect on the distribution of galactose. *Am. J. Physiol.*, 163: 70, 1950.
43. Trucco, R. E., Caputto, R., Leloir, L. F. and Mittelman, N. Galactokinase. *Arch. Biochem.*, 18: 137, 1948.
44. Leloir, L. F. The metabolism of hexose phosphates. In: Phosphorus Metabolism, p. 67. Edited by McElroy and Glass. Baltimore, 1951. Johns Hopkins Press.
45. Leloir, L. F. The enzymatic transformation of UDPG into a galactose derivate. *Arch. Biochem. Biophys.*, 33: 186, 1951.
46. Schwarz, V., Golberg, L., Komrower, G. M. and Holzel, A. Metabolic observations on the erythrocytes from cases of galactosaemia. *Biochem. J.*, 59: 22, 1955.
47. Isselbacher, K. J., Anderson, E. P., Kalckar, H. M. and Kutarashi, K. Congenital galactosemia: a single enzymatic block in galactose metabolism. *Science*, 123: 635, 1956.
48. Eisenberg, F., Jr., Isselbacher, J. K. and Kalckar, H. M. Studies on the metabolism of C14-labelled galactose in a galactosemic individual. *Science*, 125: 116, 1957.
49. Schwarz, V., Golberg, L., Komrower, G. M. and Holzel, A. Some disturbances of erythrocyte metabolism in galactosaemia. *Biochem. J.*, 62: 34, 1956.
50. Platt, B. S. Human nutrition and the sophistication of foods and feeding habits. *Brit. M. J.*, 1: 179, 1955.
51. Brody, S. and Sadhu, D. P. Nutritional significance of milk with special reference to milk sugar. *Scient. Monthly*, 64: 5, 1947.
52. Anderson, E. P., Kalckar, H. M. and Isselbacher, K. J. Defect in uptake of galactose-1-phosphate into liver nucleotides in congenital galactosemia. *Science*, 125: 113, 1957.

Sporadic or Non-Endemic Familial Cretinism with Goiter*

JOHN B. STANBURY, M.D. *and* EDWARD M. MCGIRR, M.B., M.R.C.P.
Boston, Massachusetts

CRETINISM is the state characterized by retarded physical and mental development which is due to functional failure of the thyroid gland during fetal life or during the period of early postnatal growth. It occurs most frequently in regions of endemic goiter in the offspring of goitrous parents. So-called endemic cretinism is thought to be due to a deficiency of iodine during critical phases of early development, and it is almost invariably associated with goiter. In regions where dietary iodine is abundant cretinism occurs sporadically. The common type of sporadic or non-endemic cretinism is due to failure of the thyroid gland to undergo proper development, and thyroid tissue is absent or rudimentary. A goiter is present in an occasional case of sporadic cretinism because of the action *in utero* or after birth of a goitrogenic substance such as thiouracil or cobalt.

Cretins with large goiters are rarely found where there appears to be no possibility of deficiency of iodine or of the ingestion of goitrogens. Such cases have usually been familial. Studies with radioactive iodine (I-131) have greatly furthered our understanding of their pathogenesis. These studies have disclosed that the glands of these patients may be unusually avid for iodine, although other laboratory data may indicate little if any production of effective thyroid hormone. The findings in different groups of familial goitrous cretins suggest that their cretinism and goiters are due to defects in the synthesis of the thyroid hormones. Indeed, it is now possible to attribute the hypothyroidism and thyroid enlargement in some of these cretins to one of several specific and demonstrable biochemical abnormalities which have been detected at different steps in the synthesis of the thyroid hormones. There also is evidence from genetic studies that these biochemical lesions may be genetically determined.

The cases of sporadic goitrous cretinism which appeared in the literature before 1956 have recently been reviewed in detail by Lelong et al. [*1*]. It is our purpose to present the evidence for the various biochemical abnormalities so far defined and to consider the suggestion that these biochemical abnormalities are genetically determined. To make the subsequent account more easily understood by the general reader there is a preliminary summary of the general scheme of iodine metabolism.

PATHWAYS OF IODINE METABOLISM

The principal metabolic pathways of iodine are now reasonably well formulated. These are illustrated in the accompanying diagram. (Fig. 1.) Iodide and most forms of iodine are absorbed through the gastrointestinal tract as inorganic iodide and rapidly distributed throughout the extracellular fluid compartment of the body. Iodinated tyrosines and thyronines may be absorbed intact. The kidney and the thyroid remove iodide from the plasma. The relationship between the thyroid and renal iodide clearance rates, as measured with labeled iodide, determines the result of the familiar uptake test of thyroid function. Plasma iodide is trapped initially in dialyzable form in the thyroid. A concentration ratio is achieved between thyroid and plasma which may vary from twenty to one in the resting gland to several hundred to one in the hyperplastic gland. Nonetheless, the thyroid iodide is in free exchange with the iodide of the plasma. The mechanism of iodide trapping is obscure. The process is oxygen-dependent [*2*] and is doubtless enzymatically controlled, like all other biochemical processes in the thyroid.

* From the Department of Medicine of the Harvard Medical School, the Medical Services of the Massachusetts General Hospital, Boston, Massachusetts, and the University Department of Medicine, Royal Infirmary, Glasgow, Scotland.

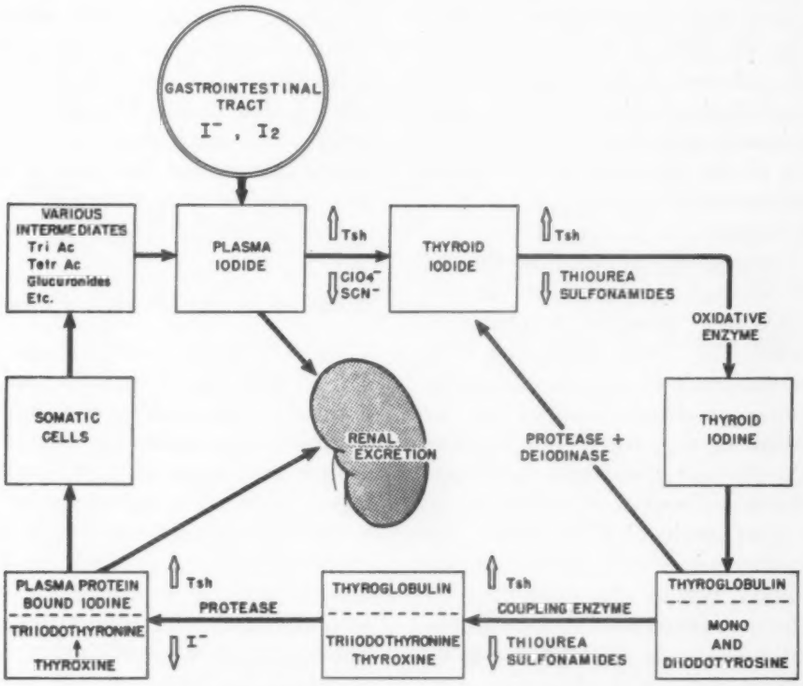


FIG. 1. The principal metabolic pathways of iodine. The open arrows pointing upward indicate stimulation and those pointing downward indicate inhibition.

The magnitude of the thyroid-to-plasma iodide ratio is governed by the thyrotropic hormone of the anterior pituitary, but the ratio is also dependent upon the quantity of stored iodine within the thyroid gland itself. Trapping of iodide is inhibited by a variety of anions such as thiocyanate and perchlorate. If these substances are given after trapping of iodide has occurred, the iodide is rapidly discharged from the gland. Iodine which has been formed into iodinated tyrosines or thyronines is not discharged in this way.

The trapped iodide is oxidized to elemental or free iodine. The oxidizing enzyme which is responsible for the process has not yet been identified with certainty, but there is evidence that a peroxidase is involved in the removal of an electron from iodide [*3*]. The oxidation of iodide is inhibited by drugs containing the thiocarbamide group, for example, thiouracil, and by many related substances. In this way they produce their well known therapeutic effect. By preventing the further metabolism of iodine, these drugs enable one to observe and study the process of iodide trapping.

The iodine liberated by oxidation of iodide reacts almost instantaneously with tyrosyl residues, which are probably in peptide linkage, to form monoiodotyrosine and diiodotyrosine. Studies of the rate of incorporation of I-131 into the various amino acids found in the thyroid gland have confirmed the sequential build-up of monoiodotyrosine, diiodotyrosine and thyroxine; higher specific activities are found earlier in monoiodotyrosine than in diiodotyrosine and in diiodotyrosine than in thyroxine [*4,5*]. A tyrosine iodinase, which has been described by Fawcett and Kirkwood [*6*], may accelerate the iodination of tyrosine. The site of this organic binding is uncertain. It appears that intact cells are required for the efficient iodination of thyroglobulin, but it is not clear whether the iodination occurs within the cells (and the iodinated protein is then secreted as such into the colloid) or whether it occurs in the colloid at the cell surface.

Iodinated tyrosyl residues may be stored as such in the thyroglobulin of the colloid or they may condense to form iodinated thyronines, of which thyroxine and triiodothyronine are the best known [*7,8,9*]. The available evidence favors the hypothesis that the condensation reaction for thyroxine involves coupling of two molecules of diiodotyrosine and extrusion of an alanine group. Whatever the mechanism, undoubtedly it is enzymatically controlled. It may require

the presence of free iodine within the gland. The demonstration by Pitt-Rivers that antithyroid drugs related to thiourea inhibit the *in vitro* coupling of acetyldiiodotyrosine suggests that these drugs may inhibit coupling *in vivo* [*10*]. No good explanation of the nature of the coupling reaction has been forthcoming since it was first suggested by Harington and Barger [*11*].

Even more uncertainty appertains to the mechanism and site of the synthesis of triiodothyronine. Of the possible mechanisms Roche and Michel [*12*] favor either the condensation of one molecule of monoiodotyrosine with one molecule of diiodotyrosine, or the partial deiodination of thyroxine. The relative importance of the thyroid gland and the tissues as the site of the deiodination of thyroxine to triiodothyronine also awaits clarification.

The iodinated thyronines may be secreted directly into the blood, but for the most part are stored away in the thyroid colloid as thyroglobulin. Thyroglobulin is slowly broken down by proteases and peptidases, and thyroxine and triiodothyronine are released for transfer across the epithelial cells to the blood directly or through the lymphatics [*13*]. In the process of thyroglobulin degradation, mono- and diiodotyrosine are also released, but their iodine is rapidly recovered for reutilization by enzymatic deiodination [*14*]. If by chance mono- or diiodotyrosine should escape into the peripheral blood, it is likely to be rapidly degraded to iodine in many tissues [*15–17*].

Thyroxine in plasma is bound to a protein which has an electrophoretic mobility close to α_2-globulin. A small fraction is also associated with albumin. Triiodothyronine is similarly but more loosely bound in the plasma, and is also found in association with other proteins. These hormones impinge upon the peripheral cells where they produce their characteristic stimulation of the metabolic rate and are in turn degraded to release iodide back to the plasma for re-entry into the iodide pool.

Metabolic pathways of uncertain significance have not been shown in Figure 1. There is an appreciable secretion of iodide into the saliva, bile, and into the gastric juice. Glucuronides of thyroxine and triiodothyronine are formed and secreted into the bile by the liver [*18,19*]. It has also been shown that the acetic acid derivatives of thyroxine and triiodothyronine are formed by the kidney and brain [*20*]. The studies of Roche et al. [*21*] indicate that there are two major metabolic pathways for thyroxine and triiodothyronine. One of these is sequential deiodination, and the other, oxidation of the alanine side chain through the pyruvate to the acetic acid analogues. A detailed analysis of iodine metabolism has been given by Riggs [*22*] and more recently a review by Berson has appeared in this journal [*23*].

TYPES OF CRETINISM DUE TO METABOLIC ERRORS

If, as has been indicated, the sequential steps in the synthesis of thyroid hormones are enzymatically controlled, one might expect that cretinism with goiter could result from primary failure at any one of these essential steps. Three types of familial non-endemic cretinism with goiter have been described in which the defect has been more or less defined. In one type, the defect is a failure to utilize trapped iodide. In the second, mono- and diiodotyrosine residues are not coupled with sufficient speed to insure an adequate supply of thyroid hormones. Subjects in a third group are unable to conserve iodine by deiodination of mono- and diiodotyrosine. These types will now be described in detail. Other groups with other metabolic errors doubtless await definition.

Cretinism from Failure of Organic Iodine to Form. In 1949 a sixteen year old girl was seen by one of us at the Thyroid Clinic, Massachusetts General Hospital, Boston, because of difficulty in breathing [*24*]. She was one of seven siblings, four of whom, including the patient, were goitrous cretins. The oldest cretin had had a thyroidectomy in 1942 for tracheal compression, and had a recurrence of thyroid enlargement which required a second thyroidectomy in 1956.* The parents were reputed to be half American and half Indian and were first cousins on the Indian side of the family.

The patient's development had been normal for a period of six months. Thereafter it had been retarded. Thyroid medication had been administered irregularly since the age of two. At about the age of seven, a mass had been noticed in the neck. This mass had slowly increased in size.

She was dwarfed and grossly retarded mentally. She was unable to walk and could utter only a few gutteral sounds. Bone age by Todd standards was thirteen years and nine

* Mono- and diiodotyrosine deshalogenase activity was demonstrated in thyroid tissue received at the time of the second thyroidectomy.

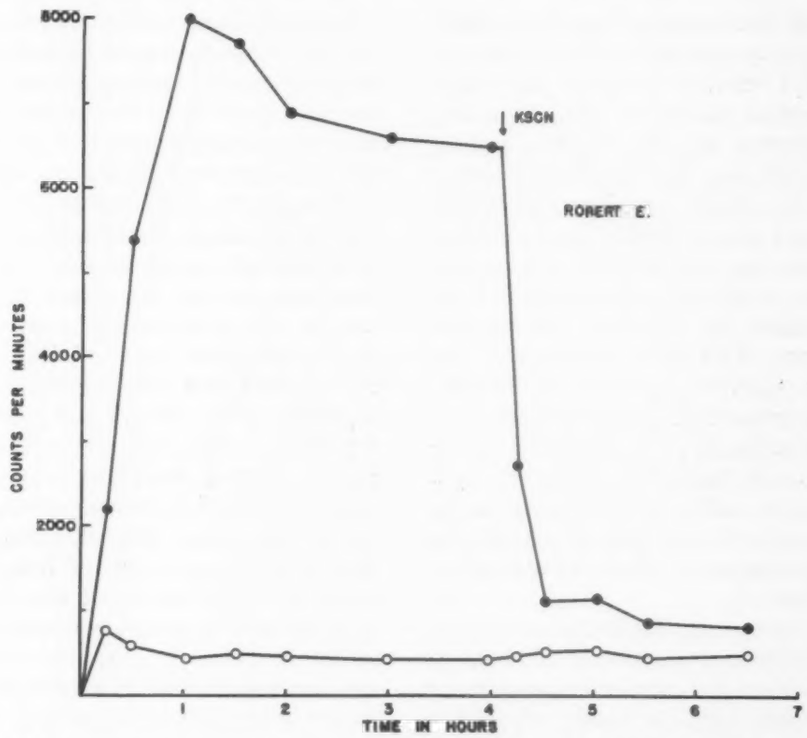


FIG. 2. The uptake and release of radioactive iodine in the thyroid gland of a patient with cretinism and goiter. The upper curve was recorded over the thyroid gland and the lower curve over the mid-back. At the arrow two gm. of potassium thiocyanate were administered orally. (Reprinted by permission of the *J. Clin. Endocrin. & Metab.*, 10: 1471, 1950.)

months. She presented the classic features of cretinism, details of which have already been published [*24*]. In addition, there was a very large nodular goiter, over which a bruit and thrill were present.

The concentration of the protein-bound iodine in the serum was 1.0 μg. per 100 ml. Microscopically, the gland, which was removed at operation, revealed intense hyperplasia in most areas, although some areas showed atrophy and replacement fibrosis. Only minute amounts of iodine were detected chemically in the gland.

When labeled iodine was given orally a large fraction of it accumulated with remarkable rapidity in the thyroid. Maximal values were observed in the gland within two hours. (Fig. 2.) When the accumulation had reached a plateau the patient was given an oral dose of 2.0 gm. of potassium thiocyanate. Immediately there was a striking and rapid disappearance of the labeled iodine from the gland. Only a small fraction of the accumulated iodine remained. Control studies were made on one of the normal sisters. The labeled iodine accumulated in her gland at a normal rate, and it was not discharged upon the administration of thiocyanate.

During subsequent months, two of the goitrous and cretinous brothers of this patient were available for similar studies. One, twenty-three years old, had a thyroid gland which weighed 497 gm. when removed. The other brother was thirteen years old; his thyroid gland was estimated to be three times the normal size. In both subjects, labeled iodine accumulated rapidly in the gland, and oral administration of potassium thiocyanate caused its precipitous discharge.

Four years later the youngest of these patients was studied again [*25*]. In the intervening time the thyroid had considerably increased in size. Gland growth could not be prevented by administration of desiccated thyroid because adequate dosage made the patient a difficult behavior problem.

Detailed observations were made of the behavior of the labeled iodine after I-131 administration. The metabolism of iodine appeared to be identical with that in patients receiving full doses of the antithyroid drugs of the thio-

carbamide group. Since these drugs exert their antithyroid effect by inhibiting the transfer of iodide to tyrosyl residues through an enzymatically controlled oxidative step, it seems reasonable to suppose that the capacity to perform the oxidative step was lacking in these patients. Thus the glands contained iodide and indeed exhibited a strong avidity for iodide but no incorporation into the organic form could occur. The result of this defect at the early stage of hormone synthesis was a condition of complete thyroid deficiency. The huge goiters and the intense cellular hypertrophy and hyperplasia were viewed as compensatory phenomena.

Confirmation of the hypothesis regarding the metabolic defect was obtained by direct analysis of thyroid tissue. No iodinated compounds could be detected by methods such as chromatography which easily demonstrated them in normal or hyperplastic glands.

Patients demonstrating this particular defect in hormone synthesis are rare. Only one patient with a similar defect has been encountered by one of the authors [*26*]. A family of similar goitrous cretins has been studied by Schultz [*27*], and isolated cases have been reported by Lelong et al. [*1*] and by Rall [*28*].

Cretinism Arising from Failure of Iodotyrosines to Couple. In 1954 a twenty-five year old woman was studied [*25*]. Both she and her sister, who also had retarded development and goiter, had first been seen and studied fourteen years earlier in the Thyroid Clinic, Massachusetts General Hospital, Boston. Their parents were not related. In spite of thyroid medication their thyroid glands had continued to enlarge. The thyroid of the elder had eventually been removed because of pressure symptoms; its histologic pattern was of intense cellular hyperplasia. On several occasions normal daily excretion of I-127 had been demonstrated and extremely high thyroid uptake of I-131 had been observed in both.

The patient showed residual stigmas of cretinism. She was physically stunted, with disproportionately short extremities, and was mentally backward. Her thyroid was considerably enlarged and was multinodular. The skin and hair were coarse and dry. The serum concentration of protein-bound iodine was 6.2 and 5.4 μg. per 100 ml. on successive determinations. Three and one-half years previously it had been 3.5 μg. and more recently 5.3 μg. The basal metabolic rate had been in the normal range and had continued so.

Detailed observations were made of the kinetics of iodine metabolism after I-131 administration. From measurements of the rate of disappearance of labeled iodine from the gland and the urinary content of labeled and stable iodine before and during the administration of methimazole® (1-methyl-2-mercaptoimidazole), a drug which blocks the incorporation of iodide into organic compounds, it was possible to calculate the rate at which stable iodine was leaving the gland. This proved to be greater than could have been possible if the secretion product had been exclusively triiodothyronine or thyroxine, else the patient would have been thyrotoxic. Also, when the amount of "hormone" leaving the gland was calculated from mean values for urinary excretion of stable iodine and uptake of labeled iodine, it was found that more than 1 mg. of iodine was secreted daily. This is at least five times the normal value. It could only be assumed that a large amount of iodide or a rapidly degraded iodinated product was leaving the gland. Chromatographic study of the serum disclosed normal amounts of triiodothyronine and thyroxine in the blood, but when the entire gland was available for analysis four days after a large dose of I-131 only a trace of thyroxine could be identified after exhaustive chemical analysis. Iodine in the gland was almost entirely present as mono- and diiodotyrosine.

It is possible to formulate an hypothesis for the metabolism of iodine in this gland as follows: The primary failure was in the enzymatic coupling of iodotyrosines. Compensatory hyperplasia produced an intensely stimulated gland with a high avidity for iodide and rapid formation of iodotyrosines. These were produced in such large amounts that a small amount of iodinated thyronines was produced non-enzymatically. During the period of active growth the amount of hormone so produced did not meet the metabolic needs of the growing child so that physical stunting and mental backwardness resulted. Later, with growth of the gland, the amount produced was probably adequate. The intensely active gland mobilized and deiodinated iodotyrosines, releasing iodide for renal excretion and for reutilization by the synthetic processes of the gland. When reutilization of iodide was inhibited with methimazole, iodide was lost in increased amounts from the gland and appeared in the urine.

Direct proof of the failure of enzymatic coupling in this patient or in others is lacking.

Indeed, the presence of such an enzyme as part of the normal machinery of the thyroid cell has yet to be convincingly demonstrated, but it almost certainly exists.

It may be that among sporadic goitrous cretins the type of defect illustrated by this patient is frequent, but the difficulty of direct proof is a handicap in diagnosis. Using a somewhat different approach, Werner, Kassenaar and Bloch [*29*] have offered evidence that the metabolic defect in a four year old goitrous patient with a high thyroidal uptake of I-131 was failure of adequate coupling of iodotyrosyl residues.

Cretinism Resulting from Failure of Iodotyrosines to Deiodinate. During the period 1951 to 1953, a number of patients were observed at the Royal Hospital for Sick Children and the Royal Infirmary in Glasgow, Scotland, who were hypothyroid or cretinous and who had goiters which had a high avidity for iodide [*30*]. The uptake of I-131 was complete after only a few hours, and measurements at twenty-four hours or later showed that a large fraction of the accumulated labeled iodine had already left the gland. Chromatographic analysis of the serum of one of these patients, who was a member of a group of itinerant tinkers in whom goitrous cretinism was known to be common (*vide infra*), failed to disclose thyroxine, but the presence of a butanol extractable compound was detected. Although it was not identified at this time the possibility that it was diiodotyrosine was entertained.

In 1955 a twenty-seven year old man entered the ward of the Academisch Ziekenhuis, Leiden, because of an enlarging goiter and symptoms of tracheal obstruction [*31*]. Enlargement of the thyroid had been noticed at birth. Thyroid medication was omitted four years prior to entry into the hospital. The goiter had relentlessly increased in size. Early development was said to have been slow but the patient achieved normal stature and mentality. His parents were unrelated. He had a normal older brother. A younger brother, who had a goiter at birth and who was severely retarded both physically and mentally, was also subsequently available for study.

The patient was a tall balding man who was slow of speech and movement. The skin was pale and cool. The thyroid was multinodular and several times the normal size. Routine examinations of blood and urine were normal except for a mild anemia. A determination of the basal metabolic rate was minus 29 per cent. The serum cholesterol concentration was 380 mg. per 100 ml., and protein-bound iodine concentration was 0.5 μg. per 100 ml.

When an oral dose of I-131 was given the labeled iodine was found to accumulate rapidly in the thyroid; within seventy minutes 74 per cent was in the gland. There was a rapid decline in the blood concentration of labeled iodine but after seventy minutes the labeled iodine began to leave the thyroid and to appear in the blood as a component which was partially precipitable with trichloracetic acid. By forty-eight hours only 25 per cent of the administered dose remained in the gland, and a serum concentration of protein-bound I-131 of approximately 0.5 per cent per liter had been reached. Subsequently, the values of labeled iodine in the serum and in the gland slowly fell.

Chromatographic analyses were made of serum at various times after administration of labeled iodine. There was increasing prominence of two spots corresponding to mono- and diiodotyrosine during the first several hours, but by seventy-two hours these had largely disappeared. Small amounts of labeled iodine were found in zones corresponding to thyroxine and triiodothyronine. Analysis of the gland received at thyroidectomy showed that approximately equal and small amounts of triiodothyronine and thyroxine were present. Most of the labeled iodine in the gland was mono- and diiodotyrosine.

Subsequently, additional observations were made on the patient, his brother, and on an unrelated twelve year old girl with short stature, mental deficiency, and a large nodular goiter [*32*]. Mono- and diiodotyrosine were noted in the serum of all three after administration of labeled iodine. Triiodothyronine and thyroxine were noted only in the first patient. Two additional zones of labeled iodine were regularly found in the serum of the last two patients. These were not identified.

Detailed chromatographic study of urine specimens was possible in the last two patients. Mono- and diiodotyrosine were found in serially collected urine specimens and, in addition, two unidentified zones of labeled iodine were noted. One of these moved near the solvent front in chromatograms developed in a butanol acetic acid solvent system, and may have been degradation products of labeled iodotyrosines. The other spot remained near the origin and was readily hydrolyzed by dilute hydrochloric acid to monoiodotyrosine. This, in addition to the fact

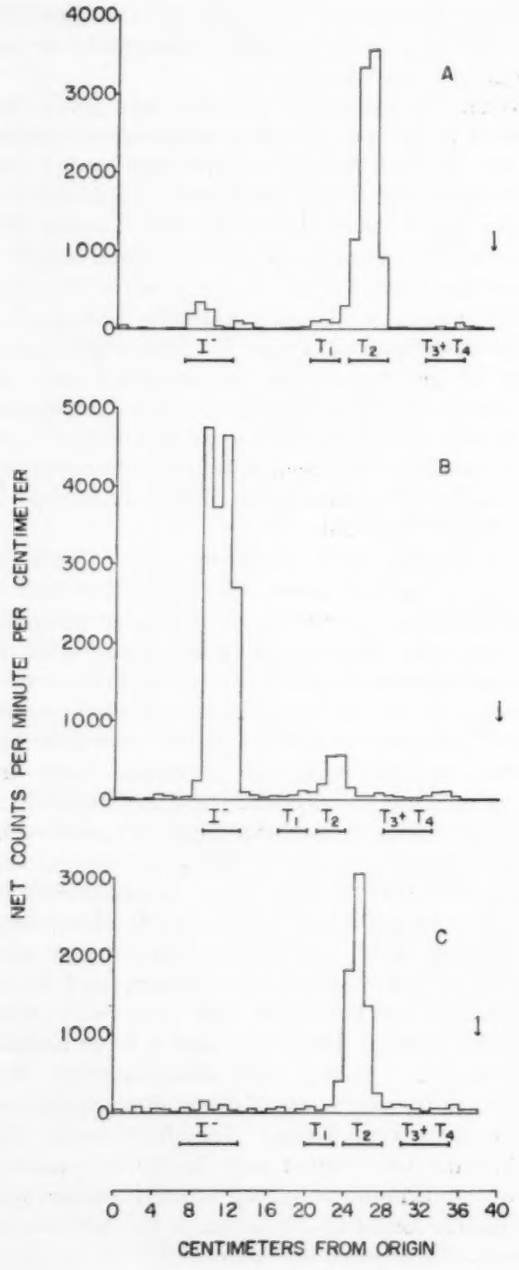


FIG. 3. Chromatographic records to show the metabolism of diiodotyrosine. Chart A is the chromatogram of the injected material. Chart B is a chromatogram of the urine of a normal subject, collected one hour after intravenous administration of I-131-labeled diiodotyrosine. Chart C is the chromatogram of the urine of a twelve year old goitrous female cretin one hour following administration of labeled diiodotyrosine. The bars correspond to the stained carriers on the chromatogram. I^-: iodide. T_1: monoiodotyrosine. T_2: diiodotyrosine. T_3 and T_4: triiodothyronine and thyroxine. Arrow points to the solvent front. (Reprinted by permission of the *J. Clin. Endocrin. & Metab.*, 16: 1522, 1956.)

that there was as much or more monoiodotyrosine present in the specimen, indicated that the principal product of these glands was monoiodotyrosine.

Observations made on these three patients after intravenous administration of I-131 labeled diiodotyrosine indicated that they were entirely unable to remove iodine from the benzene ring. (Fig. 3.) Control studies in fifteen normal subjects or patients with various diseases [*33*] indicated that less than 4 per cent of the dose was excreted unchanged in the urine in four hours, and not more than 18 per cent of the labeled iodine in any specimen of urine was present as diiodotyrosine. In the three cretins, the labeled diiodotyrosine was excreted unchanged in large amounts. No free labeled iodide was excreted except that which could be accounted for by the small amount of free labeled iodide which contaminated the injected labeled diiodotyrosine. More than 60 per cent of the administered labeled diiodotyrosine appeared unchanged in the urine within the first twenty-four hours. These observations were not significantly altered by thyroidectomy or by administration of desiccated thyroid or iodide.

Certain differences were observed after administration of labeled monoiodotyrosine. A small but detectable fraction of labeled L- or D,L-monoiodotyrosine was deiodinated, but a large fraction was excreted unchanged. By contrast, in normal subjects both optical isomers deiodinated rapidly. After administration of both L- and D,L-monoiodotyrosine, two unidentified zones of labeled iodine were seen in chromatograms of urine in addition to the iodide and monoiodotyrosine spots. These were indistinguishable from the two zones seen after ingestion of I-131 in these patients. Again, hydrochloric acid hydrolysis of the zone near the origin yielded monoiodotyrosine, indicating that this substance was in all probability a simple ester of the amino acid.

Several close relatives of this twelve year old girl were available for study. Her mother, sister, two aunts and a first cousin all had thyroid disease. All had nodular goiter clinically, except for one aunt who was said to have had Graves' disease. None had any findings suggestive of hypothyroidism and all had normal serum concentrations of protein-bound iodine. Each was studied with labeled diiodotyrosine intravenously. In all there was evidence of deiodination, but when the results were compared with

the normal group it was seen that the deiodination was significantly less rapid than in the control group of patients.

The deiodinating capacity of thyroid tissue removed from one of these cretins was tested by exposing labeled D,L-diiodotyrosine as substrate to tissue slices from the gland [*34*]. A series of control studies were made on various thyroids removed surgically for nodular goiter or Graves disease and on sheep and beef thyroid glands. Deiodination was readily demonstrable in all. One of the nodular goiters was tested on the same day as the gland from the cretin. No deiodination of substrate diiodotyrosine occurred when the test tissue was from the cretin.

The findings in the three cretins were considered to be consistent with the hypothesis that these patients lacked the tissue iodotyrosine deshalogenase which has been described by Roche et al. [*14*]. Since there was no mechanism to remove iodine from mono- and diiodotyrosine as they became freed from thyroglobulin, these substances escaped into the circulation. Since there was no means of removing iodine in the periphery, they were lost through the kidney. A vicious circle of iodine-want was established, resulting in a hyperplastic gland devoted to the production and secretion of hormone precursors. This formulation does not exclude the formation of some normal hormones. Small amounts of thyroxine and triiodothyronine were noted in the serum of one of them. The possibility remains that enzymatic coupling of iodotyrosine was also inadequate in these patients and that normally in some obscure way this reaction is coupled with deiodination.

This type of biochemical abnormality is probably fairly common among non-endemic goitrous cretins. More recent chromatographic studies in Glasgow [*35*] on two additional affected members of the tinker group, mentioned in the opening paragraph of this section, identified monoiodotyrosine and diiodotyrosine as well as thyroxine in the serum of one of the patients. In the autoradiographs of the chromatograms the monoiodotyrosine and diiodotyrosine zones of radioactivity were more pronounced in the six hour samples of serum; by forty-eight hours the thyroxine-triiodothyronine zones showed the greatest activity. The dose of radioactivity administered was not sufficient to permit identification of the chemical basis of the serum radioactivity in the other patient, but in the urine of both patients monoiodotyrosine and diiodotyrosine were present. There were also two unidentified spots of radioactivity in the urine chromatograms, similar in location to those noted in the Leiden cases, namely, near the origin of the strips and near the solvent fronts. It was not possible to carry out any further studies but it is extremely likely that the thyroid enlargement and the hypothyroidism which were common in this family group were due to the loss of the hormone precursors from the thyroid gland. The loss of these precursors was considered to be due to deficiency of the enzyme deshalogenase. Comparison of the Glasgow findings with those reported from Leiden supports the opinion that the metabolic abnormalities exhibited by the two groups of patients are identical. Horst [*36*] has recently reported two patients who appear to be similar.

Costa et al. [*37*] have chromatographic evidence of diiodotyrosine in the serum of three cretins from an endemic region of northern Italy. These may be variants of the disease. One of us has observed a twenty-four year old man with cretinism and no detectable goiter (he had received full doses of thyroid for most of his life) who had excreted almost but not quite all of a dose of labeled L-diiodotyrosine unchanged in the urine, yet on two occasions L-monoiodotyrosine was almost completely deiodinated [*38*]. The latter finding was unchanged by administration of 2 gm. of tyrosine. This was given on the hypothesis that the patient was utilizing an alternative pathway for disposal of monoiodotyrosine, and that by saturating it with tyrosine one could bring to light a primary deshalogenase defect. Blizzard [*39*] has observed a similar patient with no capacity to deiodinate diiodotyrosine, but with good deiodination of monoiodotyrosine.

Cretinism from Other Causes Which Are Probably Metabolic. The biochemical abnormalities described may reasonably be claimed to be adequately established, but it appears to be only a matter of time until blocks at other stages of synthesis of the hormone are discovered. Indeed, already a number of non-endemic goitrous cretins have been described who do not fit into the preceding categories. Di George and Pachkis [*40*] have observed one patient in whom protein-bound labeled iodine which was being discharged into the blood was not extractable with acid butanol. The authors have also seen two mentally retarded sisters, both hypothyroid and goitrous, who had an unidentified iodinated component in the serum. It was possible to

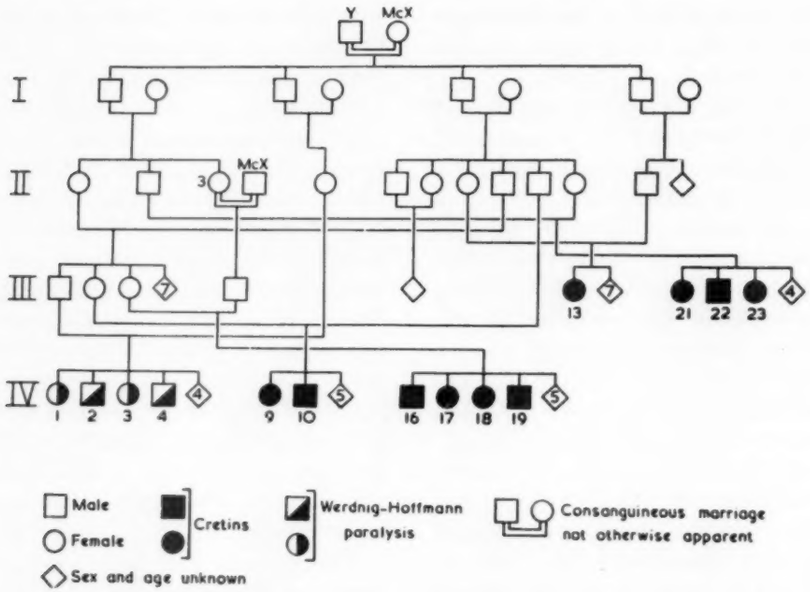


FIG. 4. Family tree of Scottish tinkers, showing appearance of goitrous cretinism and Werdnig-Hoffmann paralysis. (Modified from HUTCHISON, J. H. and MCGIRR, E. M.: *Lancet*, 1: 1035, 1956.)

identify thyroxine and a little triiodothyronine in the blood but not iodinated tryosines. Acid butanol extracted only 50 to 60 per cent of the labeled iodine from the acidified serum, whereas normally with the same technic 85 per cent or more was extracted. Unfortunately, hydrolysis experiments were not carried out and nothing further is known about the specific biochemical defect of these patients. We have also seen several other cretins who seemingly do not fit into any of the categories which have been described up to the present time.

HEREDITARY ASPECTS

Childs and Gardner [*41*] recently sought evidence of a hereditary tendency in the common type of sporadic cretinism. They found that, although there seemed to be such a tendency, a familial prevalence was distinctly uncommon. Consanguineous mating was barely more common among the parents of sporadic cretins than among the parents of normal children. In marked contrast are the findings in the more rare sporadic goitrous cretinism, in which familial prevalence and consanguinity of the parents are common. The familial tendency in this condition was recognized by Osler [*42*], who also commented on the occurrence of consanguinity. The older relevant literature has been reviewed by Gates [*43*].

The cases of sporadic goitrous cretinism of any of the types which have been reasonably well studied from the metabolic point of view in the last few years have been analyzed by Lelong et al. [*1*]. Information about sixty-one patients involving thirty-nine families is now available. Eleven families were responsible for thirty-six of the cases. Seventeen occurred in six families, in which the parents were consanguineous, and ten of these were members of four interrelated families. It is obvious that goitrous cretinism in its various forms collectively shows a high familial incidence both in the United States and in Europe, and that consanguineous marriages are strikingly frequent among the parents of these cretins. Accordingly, it has been postulated that the conditions may all be due to an appropriate "inborn error of metabolism," that they must be genetically determined, and that they are biochemical anomalies of the type originally described by Garrod [*44–46*].

Particular support for the theory of hereditary transmission of one form of sporadic goitrous cretinism comes from the remarkable group mentioned here briefly of itinerant tinkers located in the west of Scotland. Further details of this pedigree and of its genetic implications have been recently described [*47*]. Their family tree (Fig. 4) has been traced back for 160 years. The original male member of the family, Y,

came from Ireland and married a McX, his full cousin. A female Y (II, 3: Fig. 4) in a later generation also married a McX, her full cousin, but no further details of the McX family are available, although its members are stated to be scattered widely in Scotland. The number of intermarriages in generations II and III is astounding. It is thought to have been encouraged by the increasing isolation of these tinker folk from the other inhabitants of the country by their unique and self-demarcated mode of life.

Ten goitrous cretins are known to have appeared among thirty-one persons in four sets of siblings. In addition, there have been four cases of Werdnig-Hoffmann paralysis.

The pedigree of this family satisfies three of the criteria of simple recessive inheritance [*48*]. (1) The parents of affected persons were normal to outward appearance. (2) There was a striking familial prevalence: in three of the four sets of siblings there was more than one cretin. In recessive inheritance the ratio of affected to normal is 1:3. The ratio is exceeded in this family, but the total number of normal siblings was probably not known accurately. (3) All affected children were the offspring of consanguineous marriages.

Two additional criteria of autosomal recessive inheritance are not satisfied. These are: (4) The offspring of marriages of affected persons with normal persons are usually normal. (5) The offspring of marriages of two affected persons are all affected.

In view of the predilection for consanguineous mating shown by this group of tinkers, it may be that with adequate substitution therapy this position may alter with time.

The information provided by the family tree even at present strongly supports the opinion that the enzyme defect in these tinker patients is genetically determined and that it is transmitted by a single autosomal recessive gene. The combined results of the biochemical and genetic studies made in the Dutch and Scottish cases suggest that a single recessive gene controls the presence or absence of a single deiodinating enzyme [*49*]. The absence of the enzyme explains all the clinical features observed in these particular goitrous cretins.

It is a reasonable assumption that the cases of goitrous cretinism described in Scotland and in Holland with frankly abnormal clinical features, namely, thyroid enlargement and hypothyroidism, and biochemical evidence of a gross metabolic defect were homozygous. In one of the Dutch cases, the patient had several relatives who had a detectable but less obtrusive biochemical abnormality and little or nothing in the way of clinical features apart from goiter. They were, in all likelihood, heterozygous. If the disease is the result of a single enzyme defect and if the enzyme is dependent upon a single recessive gene, the gene must be incompletely recessive for defects to occur in the heterozygous state. Such an hypothesis is in keeping with the modern genetic conception that a defect transmitted by an autosomal gene as a recessive, in the heterozygous state, may produce minor but detectable metabolic abnormalities although the florid symptomatology of the homozygous state is naturally lacking [*50*].

SUMMARY

Biochemical Aspects. Sporadic cretinism with goiter may be explained on the basis of arrest at any stage of the synthesis of the thyroid hormone. Three mechanisms have already been described.

1. The thyroid gland cannot utilize trapped iodide. It is able to concentrate trapped iodide from the serum but it cannot produce iodotyrosines from tyrosyl residues and iodide, presumably because of lack of an oxidative enzyme.

2. The thyroid gland cannot couple iodotyrosines into iodothyronines with sufficient speed to secure an adequate supply of hormone. The presumption is that this defect is due to failure of enzymatic coupling, but proof is lacking.

3. The hormone precursors mono- and diiodotyrosine escape from the thyroid gland into the blood and then into the urine because of lack of the enzyme, deshalogenase. The result is a leak of iodotyrosines of sufficient magnitude to cause serious iodine deficiency.

Other abnormal mechanisms occur. It appears to be only a matter of time until these other biochemical abnormalities are elucidated.

Genetic Aspects. Genetic studies support the theory of the hereditary transmission of some types of sporadic cretinism with goiter. The condition shows a high familial incidence, and consanguineous matings are strikingly frequent among the parents of cretins.

Genetic studies in a group of Scottish tinkers indicate that the biochemical anomaly there present is genetically determined and that it is transmitted by a single recessive autosomal gene.

The combined results of biochemical and

genetic studies made in Scottish and in further similar Dutch cases suggest that a single gene controls the presence or absence of a single deiodinating enzyme.

The genetics of the two other well defined but apparently unrelated biochemical lesions have not been worked out.

Acknowledgment: In the Leiden cases the patients were under the medical care of Professor Andries Querido of the Academisch Ziekenhuis; in the Glasgow cases they were under the care of Dr. James Hutchison, Royal Hospital for Sick Children. The authors are indebted to them for the opportunity to share in the investigation of their patients.

REFERENCES

1. Lelong, M., Joseph, R., Canlorbe, P., Job, J.-C. and Plainfosse, B. L'hypothyroidie par anomalie congénitale de l'hormonogenese. (Cinq observations.) *Arch. franc. pediat.*, 13: 1, 1956.
2. Slingerland, D. W. The influence of various factors on the uptake of iodine by the thyroid. *J. Clin. Endocrinol. & Metab.*, 15: 131, 1955.
3. DeRobertis, E. and Grasso, R. Peroxidase activity of the thyroid gland under normal and experimental conditions. *Endocrinology*, 38: 137, 1946.
4. Taurog, A., Chaikoff, I. L. and Tong, W. On the occurrence of monoiodotyrosine in the thyroid gland. *J. Biol. Chem.*, 178: 997, 1949.
5. Chaikoff, I. L. and Taurog, A. Symposium on the Use of Isotopes in Biology and Medicine, p. 292. Madison, 1948. University of Wisconsin Press.
6. Fawcett, D. M. and Kirkwood, S. The synthesis of organically bound iodine by cell-free preparation of thyroid tissue. *J. Biol. Chem.*, 205: 795, 1953.
7. Kendall, E. C. The isolation in crystalline form of the compound containing iodine which occurs in the thyroid; its chemical nature and physiological activity. *Tr. A. Am. Physicians*, 30: 420, 1915.
8. Harington, C. R. Chemistry of thyroxine. II. Constitution and synthesis of diiodothyronine. *Biochem. J.*, 20: 300, 1926.
9. Gross, J. and Pitt-Rivers, R. 3:5:3′-triiodothyronine. I. Isolation from thyroid gland and synthesis. *Biochem. J.*, 53: 645, 1953.
10. Pitt-Rivers, R. The action of antithyroid substances on the formation *in vitro* of acetylthyroxine from acetyldiiodotyrosine. *Biochim. et Biophys. Acta*, 2: 311, 1948.
11. Harington, C. R. and Barger, G. Chemistry of thyroxine. III. Constitution and synthesis of thyroxine. *Biochem. J.*, 21: 169, 1927.
12. Roche, J. and Michel, R. Nature, biosynthesis and metabolism of thyroid hormones. *Physiol. Rev.*, 35: 583, 1955.
13. Dobyns, B. M. and Hirsch, E. Z. Iodinated compounds in the lymphatic pathways leading from the thyroid. *J. Clin. Endocrinol. & Metab.*, 16: 153, 1956.
14. Roche, J., Michel, O., Michel, R., Gorbman, A. and Lissitzky, S. Sur la deshalogenation enzymatique des iodotyrosines par le corps thyroide et sur son role physiologique. II. *Biochim. et Biophys. Acta*, 12: 570, 1953.
15. Hartman, N. Über den Abbau von Dijodtyrosin in Gewebe. *Ztschr. f. physiol. Chem.*, 285: 1, 1950.
16. Johnson, H. W. and Albert, A. The excretion and distribution of I-131 following administration of physiologic amounts of labeled iodide, diiodotyrosine, and thyroxine in the rat. *Endocrinology*, 48: 669, 1951.
17. Tong, W., Taurog, A. and Chaikoff, I. L. The metabolism of I-131 labeled diiodotyrosine. *J. Biol. Chem.*, 207: 59, 1955.
18. Taurog, A., Briggs, F. N. and Chaikoff, I. L. I-131 labeled l-thyroxine. II. Nature of the excretion product in bile. *J. Biol. Chem.*, 194: 655, 1952.
19. Roche, J., Michel, R. and Tata, J. Sur l'excretion biliare et la glycuroconjugaison de la 3:5:3′-triiodothyronine. *Biochim. et Biophys. Acta*, 11: 543, 1953.
20. Rawson, R. W. Today's thyroidologists and their beckoning frontiers. *J. Clin. Endocrinol. & Metab.*, 16: 1405, 1956.
21. Roche, J., Michel, R., Jouan, P. and Wolf, W. The recovery of 3:5:3′-triiodo-thyroacetic acid and 3:3′-diiodothyronine from rat kidney after injection of 3:5:3′-triiodothyronine. *Endocrinology*, 59: 425, 1956.
22. Riggs, D. S. Quantitative aspects of iodine metabolism in man. *Pharmacol. Rev.*, 4: 370, 1952.
23. Berson, S. A. Pathways of iodine metabolism. *Am. J. Med.*, 20: 653, 1956.
24. Stanbury, J. B. and Hedge, A. N. A study of a family of goitrous cretins. *J. Clin. Endocrinol.*, 10: 1471, 1950.
25. Stanbury, J. B., Ohela, K. and Pitt-Rivers, R. The metabolism of iodine in 2 goitrous cretins compared with that in 2 patients receiving methimazole. *J. Clin. Endocrinol. & Metab.*, 15: 54, 1955.
26. Stanbury, J. B. Cretinism with goiter: a case report. *J. Clin. Endocrinol.*, 11: 740, 1951.
27. Schultz, A., Flink, E. B., Kennedy, B. J. and Zieve, L. Exchangeable character of accumulated I-131 in the thyroid gland of a goitrous cretin. *J. Clin. Endocrinol. & Metab.*, 17: 411, 1957.
28. Rall, J. E. Personal communication.
29. Werner, S., Kassenaar, A. A. H. and Bloch, R. Personal communication.
30. McGirr, E. M. and Hutchison, J. H. Radioactive-iodine studies in non-endemic goitrous cretinism. *Lancet*, 1: 1117, 1953.
31. Stanbury, J. B., Kassenaar, A. A. H., Meijer, J. W. A. and Terpstra, J. The occurrence of mono- and diiodotyrosine in the blood of a patient with congenital goiter. *J. Clin. Endocrinol. & Metab.*, 15: 1216, 1955.
32. Stanbury, J. B., Meijer, J. W. A. and Kassenaar, A. A. H. The metabolism of iodotyrosines. II. The metabolism of mono- and diiodotyrosine in certain patients with familial goiter. *J. Clin. Endocrinol. & Metab.*, 16: 848, 1956.
33. Stanbury, J. B., Kassenaar, A. A. H. and Meijer, J. W. A. The metabolism of iodotyrosines. I. The fate of mono- and diiodotyrosine in normal sub-

jects and in patients with various diseases. *J. Clin. Endocrinol. & Metab.*, 16: 735, 1956.

34. Querido, A., Stanbury, J. B., Kassenaar, A. A. H. and Meijer, J. W. A. The metabolism of iodotyrosines. III. Diiodotyrosine deshalogenating activity of human thyroid tissue. *J. Clin. Endocrinol. & Metab.*, 16: 1096, 1956.
35. McGirr, E. M., Hutchison, J. H. and Clement, W. E. Sporadic non-endemic goitrous cretinism. Identification and significance of monoiodotyrosine and diiodotyrosine in serum and urine. *Lancet*, 2: 906, 1956.
36. Horst, W. Radiojoddiagnostik von Struma und Schilddrüsenkrebs und Untersuchungen zur Frage einer Jodfehlverwertung in deren Pathogenese. Verh. d. 4 Jahrestagung des Deutschen Zentralausschusses für Krebsbekämpfung und Krebsforschung. Edited by Dietrich, A. *Sonderbd. zur Strahlentherapie*, 34: 150, 1956.
37. Costa, A., Cottino, F., Ferraris, G. M., Marchis, E., Marocco, F., Mortara, M. and Pietra, R. Richerche sulla patogenesi del cretinismo endemico. *Medicine (Parma)*, 3: 455, 1953.
38. Litvak, J. and Stanbury, J. B. Unpublished observations.
39. Blizzard, R. Personal communication.
40. Di George, A. M. and Pachkis. (In press.)
41. Childs, B. and Gardner, L. I. Etiologic factors in sporadic cretinism An analysis of ninety cases. *Ann. Hum. Genetics*, 19: 90, 1954.
42. Osler, W. Internal secretions considered in their physiological, pathological, and clinical aspects. *Tr. Cong. Am. Physicians & Surgeons*, 4: 169, 1897.
43. Gates, R. R. Human Genetics, p. 554. New York, 1952. Macmillan Co.
44. Garrod, A. E. Inborn Errors of Metabolism. London, 1909. Oxford University Press.
45. Hutchinson, J. H. and McGirr, E. M. Hypothyroidism as an inborn error of metabolism. *J. Clin. Endocrinol. & Metab.*, 14: 869, 1954.
46. Wilkins, L., Clayton, G. W. and Berthrong, M. Development of goiters in cretins without iodine deficiency: hypothyroidism due to apparent inability of the thyroid gland to synthesize hormone. *Pediatrics*, 13: 235, 1954.
47. Hutchinson, J. H. and McGirr, E. M. Sporadic non-endemic goitrous cretinism. Hereditary transmission. *Lancet*, 1: 1035, 1956.
48. Roberts, J. A. F. An introduction to medical genetics. London, 1940.
49. Annotation: One gene: one enzyme? *Lancet*, 2: 1035, 1956.
50. Neel, J. V. and Schull, W. J. Human Heredity, p. 75. Chicago, 1954. University of Chicago Press.

Inborn Errors of Metabolism in Red Cells of Congenital Hemolytic Anemias*

T. A. J. PRANKERD, M.D.

London, England

ACCESSIBILITY has been an important factor in making red cells the center of so much metabolic study. As yet they are the only cells in which inborn errors of metabolism can be easily studied.

Metabolism in the mature red cell is predominantly glycolytic, and outside this metabolic pathway but few synthetic activities have been described. Energy gained from the anaerobic breakdown of glucose is stored in chemical form within the cell as adenosine triphosphate (ATP) (there are only trace amounts of creatine phosphate in the red cell). Such stored energy is ultimately coupled to a number of dependent reactions of which the maintenance of electrolyte fluxes and membrane structure are vital for cell survival. It has been clearly established that in the absence of glycolysis cation transport fails [*1*], as does lipid synthesis in the stroma [*2*]. Loss of cation transport leads to re-equilibration of intra- and extracellular ions with progressive swelling of the cell due to the excess osmotic pressure of the hemoglobin within the cell. This would eventually lead to hemolysis, but it has been shown that during prolonged incubation *in vitro* progressive cell swelling ceases after twenty-four hours [*3*] and that from then onwards red cells shrink. What the cause of this reversal of cell volume is remains unknown. It could result from the considerable loss of cell inorganic phosphate occurring after twenty-four hours incubation, which would lead to a marked fall in intracellular osmotic pressure [*4,5*].

Glycolysis in red cells has now been extensively investigated and defects in this process have been detected in several abnormal types of cell. In this article those defects which have been detected in patients with congenital hemolytic anemias will be discussed, and an attempt made to see how these abnormalities fit the basic principles governing inborn errors of metabolism. As yet, investigations of lipid synthesis in red cells of patients with this group of anemias have not been reported.

METHODS OF STUDY

A brief description of the methods used to study red cell metabolism is necessary so that the abnormalities detected by them may be more easily understood.

Glucose utilization and lactate production can be followed by direct chemical estimation or indirectly by the release of CO_2 from bicarbonate in the suspending medium. On incubation of red cells with ^{32}P-orthophosphate this isotope exchanges with unlabelled phosphate within the cell. The rate of exchange is dependent upon glycolysis within the cell since the isotopic phosphorus is incorporated into the cell's phosphate esters; these esters can be separated and the turnover of ^{32}P through them estimated [*5*]. By these estimations an intimate picture of red cell glycolysis can be built up [*6*].

Certain principles should be borne in mind in interpreting results. Firstly, the presence of white cells in the incubating sample may result in increased glucose consumption and abnormal amounts of certain phosphate esters. The same may be said of reticulocytes, which have a somewhat higher content of 2,3-diphosphoglycerate (2,3-DPG) than mature cells. These cells should be removed when possible by centrifuging. The maintenance of pH is also important as glycolysis increases in rate with a rise of pH, and vice versa. Differences in the methods of different investigators should be noted in comparing results.

NORMAL RED CELL METABOLISM

In normal whole blood, glucose consumption occurs at about 2.0 μM./ml. of cells/hour and is not significantly altered by reticulocyte concentrations up to 20 per cent provided the blood is kept well oxygenated. ^{32}P-orthophosphate exchange is a logarithmic function up to equilib-

* From the Medical Unit, University College Hospital Medical School, London, W.C.1, England.

TABLE I
METABOLIC DATA IN NORMAL RED CELLS

Glucose Consumption (μM./ml. cells/hr.)	^{32}P Exchange Constant (hr^{-1})	Quantities and Relative Specific Activities (RSA) of Intracellular Phosphate Compounds after Four Hours' Incubation					
		IP		ATP		2,3-DPG	
		μg./ml.†	RSA*	μg./ml.†	RSA*	μg./ml.†	RSA*
2.2	0.24	59.4 ±9.2	300 ...	43.2 ±5.4	380 ...	166.0 ±10.1	400 ...

* Counts/min./μg. phosphorus.
† μg. phosphorus/ml. packed cells.

rium, which is reached after four hours incubation, and its rate is very constant. Intracellular ^{32}P-turnover among esters follows a reproducible pattern and is highest in the 2,3-DPG and ATP pools and lowest in the inorganic phosphate (IP) pool. From these facts one may conclude that the radioactivity of the IP pool is derived from the breakdown of more rapidly labelled esters, and that there is little or no direct exchange between the intra- and extracellular orthophosphate. In the absence of glucose ^{32}P-exchange ceases but nucleosides will act as suitable substrates in place of glucose. It has been shown that the red cell can utilize the ribose fraction of nucleosides to maintain glycolysis [*5*]. Phosphate esters can be detected in small amounts in the stroma of red cells and the turnover of ^{32}P through these appears to be slower than those separated from the whole cell. These changes are summarized in Table I.

TYPES OF CONGENITAL HEMOLYTIC ANEMIA WITH ABNORMAL METABOLISM

So far red cell metabolic abnormalities have been reported only in hereditary spherocytosis and in the miscellaneous group of congenital non-spherocytic hemolytic anemias. The author has examined red cells from patients with thalassaemia and certain hemoglobinopathies including sickle cell anemia. None of these has shown any abnormality excepting cells which have already become sickled [*4*]. Recently Carson et al. [*8*] have reported the absence, presumably congenital, of glucose 6-phosphate dehydrogenase from the red cells of Negro subjects in whom hemolytic anemia developed during treatment with primaquine.

METABOLISM OF RED CELLS IN HEREDITARY SPHEROCYTOSIS

In these cells rates of glucose consumption have been reported as normal [*3*], as has the rate of ^{32}P exchange [*9,10*]. However, the turnover of ^{32}P-orthophosphate through esters within the cell is distinctly different, for the highest activities are found in the IP pool while the activities in the 2,3-DPG and ATP pools are significantly lower than normal [*9,10*]. These findings indicate a basic intracellular defect in phosphorylation which has been confirmed by Motulsky et al. [*11*]. In many of the subjects examined the changes were found to be reversible by providing a nucleoside as an alternative substrate for metabolism. Originally the reduction in relative specific activity of the ATP and DPG fractions of these cells was thought to reflect a reduced ability on the part of the cell to phosphorylate these esters [*9*]. However, more recent analyses have indicated that the decrease in relative specific activity of these esters actually reflects an increase in phosphorylation since there is a corresponding increase in pool size [*12*]. Details of these changes can be seen in Table II.

As yet no specific enzyme defect has been located in the red cells of hereditary spherocytosis to account for the observed abnormalities. However, certain deductions can be made, and the work of Tabechian et al. [*12*] gives further clues regarding the side of the defective enzyme. These workers found that there was a difference in ^{32}P-

TABLE II

METABOLIC DATA IN RED CELLS OF HEREDITARY SPHEROCYTOSIS

Glucose Consumption (μM./ml. cells/hr.)	^{32}P Exchange Constant (hr^{-1})	Quantities and Relative Specific Activities (RSA) of Intracellular Phosphate Compounds after Four Hours' Incubation					
		IP		ATP		2,3-DPG	
		μg./ml.†	RSA*	μg./ml.†	RSA*	μg./ml.†	RSA*
2.0	0.22	43.3 ±6.0	550 ...	39.2 ±6.6	235 ...	156.1 ±10.4	310 ...

* Counts/min./μg./phosphorus.
† μg. phosphorus/ml. packed cells.

exchange between normal and hereditary spherocytosic red cells in the presence of sodium fluoride. Normal cells with NaF (10^{-3}M) showed little or no alteration in their rates of ^{32}P-exchange [*5,12*], whereas with hereditary spherocytosic cells the exchange process ceased after approximately one hour. This effect could be prevented by the addition of a nucleoside. The reverse effect was also investigated; red cells were incubated with ^{32}P-orthophosphate, washed, and reincubated in a radioactive-free medium and the gain of radioactivity in this medium measured. The presence of sodium fluoride delayed the loss of ^{32}P from normal cells but accelerated the loss from hereditary spherocytosic cells.

These abnormalities appear to be inherent defects within the hereditary spherocytosic red cell since they have been detected in the cells of patients of all ages, and persist, as do the morphologic abnormalities of the cells, after splenectomy. Since the pool of IP in such cells has a higher activity than the pools of 2,3-DPG and ATP it cannot be derived from them, as appears to be the case in normal cells, and its direct source must be either some as yet unisolated ester or possibly the extracellular ^{32}P-orthophosphate. The fact that ^{32}P-exchange can be blocked by sodium fluoride in concentrations insufficient to block exchange in normal cells suggests that Mg^{++} activated enzymes may be concerned. Correction of the abnormalities by the presence of nucleosides during incubation would suggest that this substrate functions through an alternative metabolic pathway.

Tabechian et al. have concluded that the enzymes enolase or adenosine triphosphatases are most likely to be defective in the hereditary spherocytosic cells. However, the present writer has investigated adenosine triphosphatase activity in a number of different samples of such cells, using the methods of Clarkson and Maizels [*14*], and has found it normally active. The possibility that the defect involves enolase is supported by some unpublished data of Altman and Young [*12*], who have found that the addition of phosphate to NaF-poisoned hereditary spherocytosic cells increases the degree of NaF-inhibition of ^{32}P-exchange. This is known to be a feature of the action of sodium fluoride on enolase.

Another possibility could be that 6-phosphofructokinase, another Mg^{++} dependent enzyme, is at fault, and that hereditary spherocytosic cells bypass this defect under *in vivo* conditions by utilizing reactions in the hexose monophosphate shunt. The mechanism by which nucleosides reverse the observed abnormalities in hereditary spherocytosis cells could then be explained by the conversion of ribose 5-phosphate (derived from the nucleoside) to triose phosphates. Such a pathway, by which nucleosides reverse these abnormalities, clearly could not operate however if enolase was the abnormal enzyme. Tabechian et al. have proposed in this case that phosphoenol-pyruvate is derived from ribose 5-phosphate via hydroxypyruvate.

Other chemical defects have also been described in the hereditary spherocytosic cell. Maizels [*15*] first described the low potassium

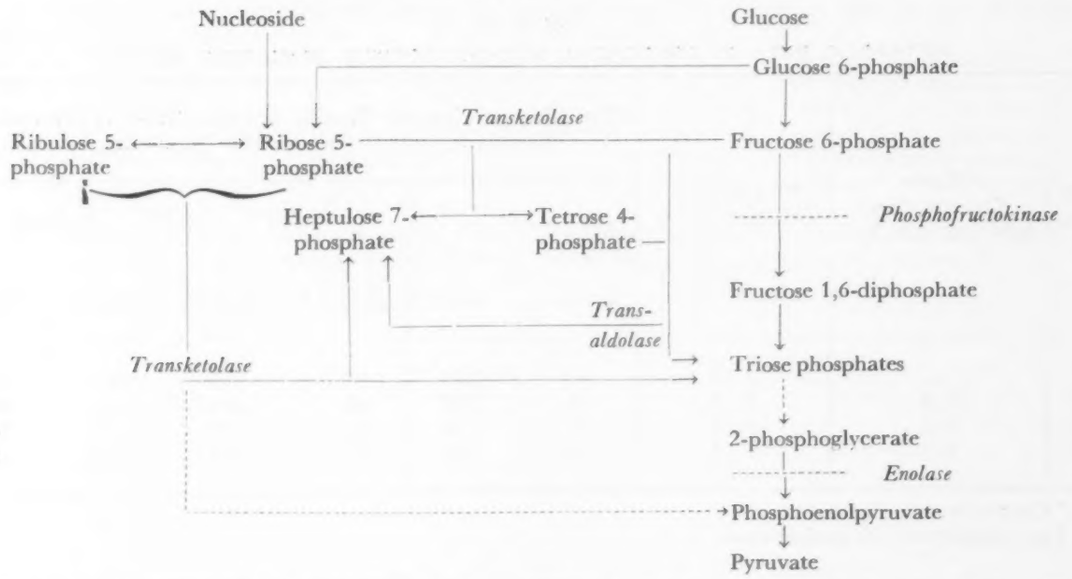


FIG. 1. Possible metabolic blocks and alternative metabolic pathways in red cells in hereditary spherocytosis. Metabolic block indicated at broken line.

content found in these cells, and Erikson et al. [*16*] have analysed the cell lipids and reported a deficiency in phospholipid with an excess of cholesterol. Both of these defects might be, however, secondary to the defect in energy metabolism.

In conclusion it would appear that there is adequate evidence indicating a defect in glycolysis in the red cell in hereditary spherocytosis. The enzyme involved in this defect has not yet been located but there are several pointers to its situation. The defects suggested would not require any alteration of the principles involved in the biochemistry of "inborn errors of metabolism." The observed changes could well be due to a single enzyme abnormality and might also be present in all cells of the body. However, the red cell, being the only cell entirely dependent upon glycolysis, would be the most vulnerable to such defects. The role this metabolic error may play in influencing the life span of the red cell in hereditary spherocytosis has been discussed elsewhere [*9,17*]. Accepting the evidence that the red cells in hereditary spherocytosis pass sluggishly through and stagnate in the splenic pulp, the hemoconcentration that ensues would create conditions in which the supply of glucose per cell is reduced, and the cells, being incapable of normal glycosis, can no longer maintain their normal chemical stores of energy, so that hemolysis ensues.

CONGENITAL NON-SPHEROCYTIC HEMOLYTIC ANEMIAS

These are a mixed group of anemias described in some detail by Dacie et al. [*18*]. In a later study [*3*] Dacie divided the cases into two groups according to whether the red cells showed increased autohemolysis, increased potassium loss on incubation and defective glucose utilization (type 2); or none of these changes (type 1). Motulsky et al. [*19*] have examined a number of cases of this group of anemias and reported normal rates of glycolysis and ^{32}P-orthophosphate exchange, but increased intracellular contents of 2,3-DPG. The present writer has examined the cells from four subjects and the results of his investigations are shown in Table III.

It will be seen that one case, (No. 2), a seven year old boy with normal red cell morphology and fragility, showed normal metabolic criteria; whereas the other three showed gross defects with low contents of ATP, deficient glucose consumption in two cases, and decreased ^{32}P-orthophosphate exchange.

It seems probable that this group of anemias can be divided into two basic groups according to whether the cells show any metabolic abnormality or not. Much further study will be required to delineate and reclassify the former group but they too are probably examples of further inborn errors of metabolism. The metabolic defects may not be specific, however, as

TABLE III

METABOLIC DATA IN CONGENITAL NON-SPHEROCYTIC HEMOLYTIC ANEMIA

Case No.	Glucose Consumption (μM./ml. cells/hr.)	^{32}P Exchange Constant (hr^{-1})	Quantities and Relative Specific Activities (RSA) of Intracellular Phosphate Compounds after Four Hours' Incubation					
			IP		ATP		2,3-DPG	
			μg./ml.†	RSA*	μg./ml.†	RSA*	μg./ml.†	RSA*
1	...	0.13	80	200	16.5	40	153	166
2	2.0	0.23	45	300	60	475	189	488
3	0.5	0.08	76	176	21	75	113	140
4	0.6	0.11	69	250	17.1	64	190	110

* Counts/min./μg./phosphorus.
† μg. phosphorus/ml. packed cells.

similar changes have been found in red cells from other conditions.

PRIMAQUINE-INDUCED ANEMIA

Mention must be made of this disorder which has been studied very recently and in which a specific defect in red cell enzymes has been located. With the use of primaquine in the treatment of malaria it was observed that a hemolytic anemia related to the administration of the drug developed in some subjects. These subjects were all Negroes [*20*]. Carson et al. [*8*] have investigated red cell metabolism in susceptible subjects and found that the red cells of those in whom hemolysis develops lack the enzyme glucose 6-phosphate dehydrogenase responsible for oxidising glucose 6-phosphate to 6-phosphogluconate. It is not known yet how this defect is related to the hemolysis induced by primaquine but it would seem to be a further example of an inborn metabolic error in erythrocytes.

It is to be hoped that this brief review has indicated an extension of Garrod's original concept into a realm of cellular pathology. Extension into the pathology of other cells may reap just rewards.

Acknowledgment: The author is very grateful to Dr. Lawrence E. Young and Dr. Kurt Altman for their comments and permission to refer to unpublished data.

REFERENCES

1. Maizels, M. Factors in the active transport of cations. *J. Physiol.*, 112: 59, 1951.
2. Altman, K. I. and Swisher, S. N. Incorporation of acetate-2-^{14}C into human erythrocyte stroma as a function of storage. *Nature, London*, 174: 459, 1953.
3. Selwyn, J. G. and Dacie, J. V. Autohemolysis and other changes resulting from incubation *in vitro* of red cells from patients with congenital hemolytic anemia. *Blood*, 9: 414, 1954.
4. Prankerd, T. A. J. Unpublished observations.
5. Prankerd, T. A. J. and Altman, K. I. A study of phosphorus metabolism in mammalian red cells. *Biochem. J.*, 58: 622, 1954.
6. Prankerd, T. A. J. The activity of enzymes in metabolism and transport in the red cell. *Int. Rev. Cytol.*, 5: 279, 1956.
7. Prankerd, T. A. J. The uptake of radioactive phosphate and its distribution amongst esters in sickled cells. *Clin. Sc.*, 14: 381, 1955.
8. Carson, P. E., Flanagan, C. L., Lakes, C. E. and Alving, A. S. Enzymatic deficiency in primaquine sensitive erythrocytes. *Science*, 124: 484, 1956.
9. Prankerd, T. A. J., Altman, K. I. and Young, L. E. Abnormalities of carbohydrate metabolism of red cells in hereditary spherocytosis. *J. Clin. Investigation*, 33, 957, 1954.
10. Prankerd, T. A. J., Altman, K. I. and Young, L. E. Abnormalities of carbohydrate metabolism of red cells in hereditary spherocytosis. *J. Clin. Investigation*, 34: 1268, 1955.
11. Motulsky, A. G., Giblett, E., Colman, D., Gabrio, B. W. and Finch, C. A. Life span, glucose metabolism and osmotic fragility of erythrocytes in hereditary spherocytosis. *J. Clin. Investigation*, 34: 911, 1955.
12. Altman, K. I. and Young, L. E. Personal communication.
13. Tabechian, H., Altman, K. I. and Young, L. E. Inhibition of ^{32}P-orthophosphate exchange by sodium fluoride in erythrocytes from patients

with hereditary spherocytosis. *Proc. Soc. Exper. Biol. & Med.*, 92: 712, 1956.

14. Clarkson, E. M. and Maizels, M. Distribution of phosphatases in human erythrocytes. *J. Physiol.*, 116: 112, 1952.
15. Maizels, M. Anion and cation contents of normal and anemic bloods. *Biochem. J.*, 30: 821, 1936.
16. Erickson, B. W., Williams, H. H., Hummal, F. C., Lee, P. and Macy, I. G. The lipid and mineral distribution of the serum and erythrocytes in the hemolytic and hypochromic anemias of childhood. *J. Biol. Chem.*, 118: 569, 1937.
17. Young, L. E. Hereditary spherocytosis. *Am. J. Med.*, 18: 486, 1955.
18. Dacie, J. V., Mollison, P. L., Richardson, V., Selwyn, J. G. and Shapiro, L. Atypical congenital hemolytic anemia. *Quart. J. Med.*, 22: 79, 1953.
19. Motulsky, A. G., Gabrio, B. W., Brokhardt, J. and Finch, C. A. Erythrocyte carbohydrate metabolism in hereditary hemolytic anemias. *Am. J. Med.*, 19: 291, 1955.
20. Hockwald, R. S., Arnold, J. and Clayman, C. B. Toxicity of primaquine in Negroes. *J. A. M. A.*, 149: 1568, 1952.

Hypophosphatasia*

DONALD FRASER, M.D.

Toronto, Canada

WHEN Garrod [*1*] delivered his classic lectures on the inborn errors of metabolism, he grouped together with remarkable insight a number of entirely separate entities, each characterized by a permanent inherited biochemical disturbance as a common feature. In recent years it has become increasingly evident that many other metabolic diseases are genetically determined, and rightly belong to this fascinating category.

In 1948, when Rathbun [*2*] coined the term "hypophosphatasia," he was not aware that the syndrome he was describing would later prove to be one of the inborn errors of metabolism. However, subsequent studies have shown this to be the case. There is now good evidence that hypophosphatasia is a specific genetically determined metabolic disease characterized by three salient features: (1) abnormal mineralization of bone, (2) diminished alkaline phosphatase activity, and (3) increased urinary excretion of phosphorylethanolamine. Therefore, it is evident that the biochemical nature of the syndrome may be established not only by the demonstration of an enzyme deficiency but also by the detection of a chemical abnormality. Such a dual manifestation is at present unique within this category of diseases.

The syndrome is rare, and only after some nine years has sufficient information become available to justify a composite description. It has become apparent, for instance, that there may be marked variations from one patient to another in the form and intensity of the disease, a feature which is in keeping with the modern concept of genetically determined biochemical disorders. Further, there may be considerable variation in the severity of the lesions in a given patient with the passage of time; but again this is not contrary to modern teaching, for there have been no complete spontaneous cures recorded to date.

Since Rathbun's report, patients with the general features of hypophosphatasia have been described by a number of investigators [*3–16*]. Furthermore, although Rathbun is rightly given credit for establishing the syndrome as an entity, earlier authors [*17–20*] have described cases which, although incompletely investigated, are now believed to be examples of the disease. The present paper also includes information concerning three hitherto unpublished cases [*21–23*].

In all, the review includes thirty-five patients all of whom I consider to be typical examples of hypophosphatasia. I shall also comment briefly upon one atypical case [*24*]. In addition to the reports cited, the reader is referred to five reviews and discussions which have been published on the subject [*25–29*].

Certain cases which have sometimes been considered examples of hypophosphatasia have been excluded from the present review since in my opinion the recorded data do not establish the diagnosis [*30–34*].

It is not my intention in this review to deal exhaustively with the various case reports. I have summarized the available data in Table 1. In the paragraphs which follow I have attempted to set forth an integrated picture of this still mysterious syndrome.

INCIDENCE

In a disease as rare as hypophosphatasia any estimate concerning incidence is obviously pure speculation. With this understanding, our own experience will be cited. In ten years four confirmed cases of hypophosphatasia from three unrelated families have been diagnosed at The Hospital for Sick Children, Toronto. Under the prevailing circumstances almost all children with serious subacute illnesses are referred to this institution, which serves a local population estimated at 2,000,000 persons. On the basis of the present birth rate in Ontario this population

* From the Department of Paediatrics, Faculty of Medicine, University of Toronto, and The Research Institute, The Hospital for Sick Children, Toronto, Canada.

is considered to have produced approximately 500,000 infants during the past ten years. If one accepts the assumptions mentioned, the incidence of hypophosphatasia during the latter period was approximately 1:100,000 live births in the Toronto area.

In the present series, twenty-one cases were females and fourteen were males.

GESTATION

Although hypophosphatasia is an inborn error of metabolism, and although skeletal lesions developed *in utero* in some cases, there have been no obvious disturbances in the course of pregnancy. Most of the reported pregnancies reached term, and the disturbances mentioned by Rathbun and Chown were minor in degree.

AGE OF ONSET

There is radiographic proof that the bone lesions of hypophosphatasia may occasionally develop *in utero* (Case 3). Certain circumstantial evidence suggests that in other cases lesions may begin to develop (or at least may become much more severe) at the time of birth (Cases 9 and 10). In addition, it has been shown that occasionally bone lesions may not become detectable until several months after birth. In the latter patients it is probable from theoretical considerations that a biochemical lesion is present at the time of birth in all cases. In this regard one patient is known to have had no clinical or radiologic evidence of hypophosphatasia until the age of nine months although at birth the serum alkaline phosphatase level had been found to be markedly reduced (Case 19, sib of Case 9).

THE CLINICAL AND RADIOLOGIC MANIFESTATIONS

In describing the clinical manifestations of hypophosphatasia it is practical to divide the cases arbitrarily into three groups based upon the estimated time of development of demonstrable bone lesions: Group I: Infants in whom lesions were present at birth or within the first six months of life (Cases 1 to 16). Group II: Children in whom lesions gradually became apparent after the age of six months (Cases 17 to 31). Group III: Patients in whom hypophosphatasia was first diagnosed in adult life (Cases 32 to 35).

In general, the patients in group I were severely affected and had a poor prognosis; those in group II and group III were less severely affected and the outlook was correspondingly better. It is not known what determines the severity of a given case. However, it should be noted that when sibs have been affected (Cases 2 and 3; 4 and 5; 6 and 7; 12 and 13; 22, 23 and 24; 30 and 31), the age of onset, severity and clinical course have usually been almost identical. As the only exception to this observation the marked dissimilarity in the clinical features of the sibs reported from this center (Cases 9 and 19) should be noted.

Group I

Infants in whom lesions were present at birth or within the first six months of life (Cases 1 to 16)

General. All the infants in group I were found upon examination to have extensive skeletal lesions. However, it was usually because the infant had acute generalized symptoms and signs that the parents sought medical advice. The infants presented with anorexia, irritability, persistent vomiting, mild pyrexia of undetermined cause, and occasionally mild anemia and dehydration. Although even the severely affected children had normal weights at birth, marked failure in linear growth and weight gain had become evident before six months of age. Blue scleras were observed in three cases (Cases 9, 10 and 33) and mild skin pigmentation was mentioned, without comment, in two cases (Cases 9 and 10). The more severely affected infants also suffered from unexplained convulsions (Cases 1, 2, 3, 4, 5, and 10) and repeated attacks of cyanosis and pneumonia (Cases 2, 3 and 8).

In seven cases acute symptoms were present within the first few days of life (Cases 1 to 7); in these there is no doubt that bone lesions must have been present before birth. In the others, symptoms did not make their appearance for three to six months, and it is more difficult to fix the date of onset of the skeletal lesions.

Skeletal Lesions. The bones of the skull and skeleton were extensively affected in this group of infants. Marked costochondral beading and prominence of the ends of the long bones similar to that seen in severe rickets were characteristically present (Cases 1, 2, 3, 4, 5, 7, 8, 9, 10 and 14). In some cases marked angulations and deformities, presumably due to previous fractures, were reported at a very early age. Frequently, kyphosis developed as the child grew older (Cases 2, 3, 8 9 and 15). Bone tenderness (Cases 1, 9 and 11) and hypotonia (Cases 8, 10, 11 and 18) were mentioned occasionally.

The radiographic signs of hypophosphatasia

TABLE 1

PRINCIPAL FEATURES OF REPORTED CASES OF HYPOPHOSPHATASIA

Case No.	Author(s)	Sex	Birth Weight (gm.)	Age at Onset (estimated)	Age (when last reported)	Outcome	Histologic Study	Clinical Manifestations						Serum Chemistry			Urinary Phosphorylethanolamine
								Long Bone Lesions	Cranial Abnormalities	Head Circumference	Premature Dental Exfoliation	Skeletal Growth	Renal Lesions and/or Renal Insufficiency	Serum Calcium mg./100 ml. (normal 9.5 to 11.5)	Alkaline Phosphatase		
															Method*	Value	
							Group I										
1	Rathbun [2]	M	4,300	Fetal	2 mo.	Dead	Autopsy	+	+	↓	..	↓	+	10.9 to 13.6	BLB	0 to 0.7	..
2	McCance et al. [13] (Case 1)	F	2,600	Fetal	3 mo.	Dead	Autopsy	+	+	.....	..	↓	+	14.4	....	.....	..
3	McCance et al. [13] (Case 2)	M	3,500	Fetal	5 mo.	Dead	Autopsy	+	+	↓	..	↓	+	11.3	Bod	0.6 to 1.2	+
4	Chown [18] (Case 1)	F	3,200	Fetal	3 mo.	Dead	Autopsy	+	..	↓	..	.....	+	15.5 to 15.8	....	.....	..
5	Chown [18] (Case 2)	F	3,400	Fetal	6 mo.	Dead	Autopsy	+	+	↓	..	↓	+	14.3 to 18.4	....	.....	..
6	Kubatsch [19] (Case 1)	M	3,750	Fetal	7 mo.	Dead	.....	+	+	.....	..	.....		.....	....	.....	..
7	Kubatsch [19] (Case 2)	F	3,500	Fetal	6½ mo.	Living	.....	+	+	Normal	..	.....		11.6	....	.....	..
8	Engfeldt et al. [8]	F	4,200	Fetal	10 mo.	Dead	Autopsy	+	+	↓	..	↓	+	9.5 to 14.0	BB	3.3 to 4.0	..
9	Fraser et al. [16,40,51] (Case 1)	F	2,700	Perinatal	2½ yr.	Living	Biopsy	+	+	↓	..	↓	+	10.7 to 15.0	KA	3.0 to 5.6	+
10	Schlesinger et al. [9,10] (Case 1)	F	3,400	Perinatal	2 yr.	Living	Biopsy	+	+	↓	..	↓	+	10.0 ot 14.9	KA	2.0 to 9.0	+
11	Anspach et al. [20] (Case 2)	F	.....	3 mo.	4 yr.	Living	.....	+	+	.....	..	↓	+	11.7 to 16.2	Bod	1.2, 1.5, 12.0	..
12	Sobel et al. [7] (Case 3)	M	.....	3 mo.	8 mo.	Dead	.....	+	..	.....	..	.....	..	↑	BLB	0.2 to 0.9	..
13	Sobel et al. [7] (Case 4)	F	.....	3 mo.	8 mo.	Dead	.....	+	..	.....	..	.....	..	↑	BLB	0.5 to 1.6	..
14	Laxdal [23]	F	.....	3 mo.	1 yr.	Dead	.....	+	+	.....	..	.....	..	Normal	Bod	3.3	+
15	Schlesinger et al. [10] (Case 2)	F	3,900	6 mo.	2 yr.	Living	.....	+	+	Normal	..	.....	+	10.7 to 10.8	KA	3.0 to 7.3	..
16	Scaglione et al. [15]	F	.....	.....	4 yr.	Living	Biopsy	+	+	.....	+	↓	..	Normal	Bod	0.2 to 1.1	+
							Group II										
17	Illingworth et al. [12]	M	4,100	5 mo.	4 yr.	Living	.....	+	0	.....	+	Normal	0	10.8 to 11.5	JK	4.0 to 5.5	..
18	Sobel et al. [7] (Case 5)	F	.....	6 mo.	9 mo.	Living	.....	+	..	.....	+	.....	..	Normal	BLB	0.7	..
19	Fraser et al. [16] (Case 2)	F	3,500	9 mo.	18 mo.	Living	.....	+	0	Normal	+	Normal	0	10.6 to 13.2	KA	3.2 to 5.2	+
20	Fraser et al. [16] (Case 3)	F	1,900	10 mo.	10 yr.	Living	.....	+	0	Normal	+	↓	0	10.0 to 11.5	KA	2.0	+
21	Felder et al. [11]	M	3,500	8 mo.	19 mo.	Living	.....	+	0	Normal	+	↓	0	11.2 to 11.9	Bod	0.1 to 0.4	..
22	Clausen [6] (Case 1)	F	.....	10 mo.	12 yr.	Living	.....	+	0	.....	+	↓	..	Normal	BLB	0.8	..
23	Clausen [6] (Case 2)	F	.....	10 mo.	10 yr.	Living	.....	+	0	.....	+	Normal	..	.....	.....	.....	..
24	Clausen [6] (Case 3)	M	.....	10 mo.	6 yr.	Living	.....	+	0	.....	+	Normal	..	.....	.....	.....	..
25	Sobel et al. [5,7] (Case 1)	F	3,000	16 mo.	3½ yr.	Living	Biopsy	+	0	.....	+	Normal	0	9.8 to 10.3	BLB	0.8 to 1.6	..

26	Sobel et al. [7]	M	.....	20 mo.	1 yr.	Living	.....	+	..	.....	+	.....	..	Normal	BLB	0.5	..
	Case 2																
27	Schlesinger et al. [10]	F	.....	2 yr.	3 yr.	Living	.....	+	+	.....	0	.....	+	12.1	KA	2.4	..
	(Case 3)																
28	Hühne et al. [17]	M	3,000	3 yr.	5 yr.	Living	.....	+	0	.....	..	↓	0	10.0	.....	.....	..
29	Harpur [27]	F	.....	Childhood	6 yr.	Living	.....	+	+	.....	+	↓	+	11.5	BLB	0.1	0
30	Schneider et al. [3]	M	.....	Childhood	15 yr.	Living	.....	+	..	.....	..	.....	0	12.0	Bod	<1	..
	(Case 1)																
31	Schneider et al. [3]	M	.....	Childhood	18 yr.	Living	.....	+	..	.....	..	.....	0	12.0	Bod	<1	..
	(Case 2)																
									Group III								
32	Dent [35,43]	F	.....	Childhood	40 yr.	Living	.....	Bone fragility	0	.....	..	↓	0	10.0	KA	2.9	+
33	Macey [4]	M	.....	Childhood	36 yr.	Living	.....	Bone fragility	+	.....	..	.....	..	10.3	?	0.4 to 0.7	..
	(Case 1)																
34	Macey [4]	M	.....	Childhood	Adult	Living	.....	.....	..	.....	..	.....	..	.....	?	<1	..
	(Case 2)																
35	Henneman [22]	M	.....	Adult	44 yr.	Living	Biopsy	Bone fragility	0	.....	..	↓	0	9.3 to 9.7	Bod	0.1 to 3.6	+

* BLB = Bessey, Lowry, Brock
KA = King and Armstrong
Bod = Bodansky
HB = Buch and Buch
JK = Jenner and Kay

are essentially the result of defective calcification in pre-osseous cartilage and in recently deposited bone matrix. Longitudinal growth of bone takes place, but at a reduced rate, and little or no calcium is deposited at the disrupted zone of provisional calcification or in the osteoid which accumulates in the metaphyses.

It is generally conceded that the resultant radiographic picture is characteristic. (Fig. 1.) There was nearly always complete disappearance of the zone of provisional calcification (Cases 1, 2, 3, 7, 8, 9, 10 and 11) and little or no bone mineral was visible in the region extending from the epiphyseal cartilage occasionally as far as 1 cm. into the diaphysis. In this region a shadow slightly less radiolucent than the surrounding soft tissues was frequently observed which conformed to the shape of what would normally have been metaphyseal bone. Occasionally, longitudinal tongues of completely uncalcified tissue were reported extending into this zone from the region of the epiphyseal plate (Cases 1 and 9).

In most radiographs a gradual transition was observed from the uncalcified metaphysis to relatively normal diaphysis, and in the latter location a frayed and frequently cupped zone of diaphyseal bone was visible (Cases 2, 3 and 11). However, as seen in Figure 1 (Case 9) and in the radiographs published by Schlesinger et al. [*10*] (Case 10), the transition was occasionally sharply demarcated and convex, beyond which, at each end of the bone, a zone of poorly calcified metaphysis projected. In Case 9 it was noted, at the age of five months, that the length of the relatively well calcified portion of the diaphyseal shadow in the tibia and the femur was approximately equal to the mean length of the corresponding diaphysis in newborn infants of comparable birth weight. Taken in conjunction with the history, the finding was thought by Fraser et al. [*41*] to suggest that a sudden metabolic alteration had occurred in the infant during the perinatal period.

In several severely affected children, radiographic evidence of irregular subperiosteal new bone formation was observed in the diaphyseal regions (Cases 1, 9 and 10). The calcification of the diaphyses appeared to be relatively normal but the diaphyses failed to grow normally in diameter. Definite defects in calcification were observed in the secondary epiphyseal centers in several cases (Cases 2, 3, 8, 10, 21, 30 and 31). The bone age was retarded

FIG. 1. Radiograph of the lower limb: Group I (Case 9, age five months). Note disappearance of the zones of provisional calcification, extreme defect in calcification of metaphyses and abrupt transition to relatively normal diaphysis. Subperiosteal calcification is visible. Epiphyseal centers are normal.

in some cases (Cases 2, 3, 10 and 21) but normal in others (Cases 1, 8 and 27).

Cranial Lesions. Cranial abnormalities were observed in thirteen of the cases in group I. It is probable that significant cranial lesions always occur when the infant is severely affected in early infancy. In Rathbun's patient, in whom the lesions undoubtedly developed *in utero*, the changes which occurred are representative of

FIG. 2. Radiograph of the skull: Group I (Case 9, age five months). Note wide bands of uncalcified osteoid between the poorly calcified membrane bones. Clinically, bulging fontanelle, reduced head circumference and craniostenosis were present.

the most extreme situation. The head was globular and the brain appeared to be enclosed in a soft fibrous membrane in which could be palpated four small plaques—the only vestiges of the frontal and parietal bones.

In no other reported case has there been so marked a defect in the cranial vault but practically all the infants in this group had conspicuous abnormalities in the development of the skull. In the more typical case the skull was described as soft, with a large bulging anterior fontanelle. In some cases the patient's eyes were unusually prominent (Cases 9, 10 and 11) and the scalp veins distended (Case 9).

The head circumference was recorded in nine cases in group I. Of these only two equalled the mean value for age (Cases 7 and 15). The remainder lay below the fifteenth percentile, and all but one below the third percentile.

The radiographic appearance of the skull in the severely affected patients with hypophosphatasia is both striking and characteristic. In Rathbun's case only four rudimentary plaques of calcified tissue were visible in the cranial vault. Frequently, reports of other severely affected infants (Cases 2, 3, 5, 8, 9, 10 and 15) have described between the cranial bones, wide bands which contained little or no visible bone mineral. (Fig. 2.) Because of their

location these wide radiolucent bands have often been referred to as "widened sutures." This concept is erroneous. The histologic studies of Rathbun [*2*], Chown [*18*] and McCance et al. [*13*] have proved that the tissue represented by the radiolucent bands is actually uncalcified osteoid, and not fibrous tissue such as is found in the widened suture lines of hydrocephalus. The point is an important one. Despite the radiologic appearance of the skull during this early period of infancy, it is my opinion that the occurrence of a bulging anterior fontanelle in the presence of reduced head circumference is evidence of craniostenosis, albeit of a unique type.

Premature exfoliation of primary teeth, a feature commonly present in patients in group II, occurred in only one of the patients in this group (Case 16).

Group II

Children in whom lesions gradually became apparent after the age of six months (Cases 17 to 31)

The children in group II, although suffering from the same fundamental disorder, first presented somewhat later in life, and with a considerably different and much less severe clinical picture. The youngest came under observation some time after six months of age, and lesions had developed in most of the patients before the age of two years. This group of children were quite healthy on the whole; most of them were brought for examination because of orthopedic deformities. However, the majority were also found to have lost two or more primary teeth prematurely (*vide infra*). In two children the dental abnormalities constituted the chief complaint and overshadowed the minor lesions which were subsequently detected in the long bones (Cases 17 and 19).

On examination, the children presented with varying degrees of genu valgum (Cases 17, 20, 22, 23, 24, 25, 27, 30 and 31) some of which required surgical correction. Growth retardation was not usually a conspicuous feature and the height was known to be normal in four children at the time of reporting. On the other hand, five children were stunted, two severely so (Cases 20 and 28).

Although fundamentally the same, the radiologic abnormalities were less severe than those seen in the infants in group I (Cases 17, 20, 21, 25 and 27). The zones of provisional calcification were usually not markedly disrupted, and the metaphyses were not so completely devoid of calcium. (Fig. 3.) The trabeculae near the metaphyseal regions were coarse, distorted and irregularly calcified, and although the zone of provisional calcification was usually in close proximity to the secondary epiphyseal center, it was frequently tilted, giving rise to severe angulations at the large joints. In some cases, tongues of radiolucent tissue projected from the region of epiphyseal cartilage into the diaphyses as wedges of poorly mineralized bone, although the

FIG. 3. Radiograph of the knee: Group II (Case 20, age three and one-half years). Note deficient and irregular calcification in metaphyses and tilting of the tibial epiphyseal plate, producing genu valgum. The diaphyses are bowed. Epiphyseal centers are normal.

latter were much less conspicuous than in the younger patients. Changes in the periosteum and the epiphyseal centers were minor in extent and frequently undetectable. Although bowing was often present in the long bones, the radiographic structure of the diaphyses appeared relatively normal.

In this group of fifteen patients craniostenosis was a relatively uncommon complication, and radiographs of the skull were usually normal. However, in two children many or all the cranial sutures had closed completely at an early age and the skull had acquired typical lacunar markings (Cases 27 and 29).

Group III

Patients in whom hypophosphatasia was first diagnosed in adult life (Cases 32 to 35)

Four patients have been designated as cases of adult hypophosphatasia. Cases 33 and 34 were sibs, and the sister of Case 31, although healthy, had typical chemical abnormalities and had had certain skeletal abnormalities as a child [*35*]. Three of the four patients presented after the age of twenty years because of undue fragility of the long bones. In each the serum alkaline phosphatase was found to be low. However, the only radiographic feature, apart from fractures or pseudo-fractures, was mild osteoporosis. In one patient the histologic appearance of the skull and ilium was normal (Case 35). The height was considerably below average in two cases (Cases 32 and 35) and was not recorded in the other two.

Two of the patients (Cases 34 and 35) had had no symptoms of bone disease during childhood and the question arises whether these patients form part of the general syndrome under discussion or whether they represent a separate entity. Further studies of the natural course of established childhood cases are required before the question can be answered. However, three features indicate that the manifestations described probably constitute the adult counterpart of the childhood illness. (1) In Cases 32 and 33 the patients had been considered "rachitic" as children. (2) In Case 33 the skull was dolicocephalic. (3) In Cases 32 and 35 phosphorylethanolamine was detected in the urine.

Group IV

"Atypical Hypophosphatasia"

In completing the description of the clinical manifestations of hypophosphatasia brief mention should be made of a case recently reported by Klein, Laron, Knapp and Sissman [*24*]. The patient, a male infant, presented with hypoglycemia, renal glycosuria and marked skin pigmentation, but without detectable bone lesions. The serum alkaline phosphatase levels were much reduced, and phosphorylethanolamine was present in the urine. A previous sib was said to have been similarly affected. These diversified biochemical disturbances might well be accounted for by a generalized deficiency of tissue alkaline phosphatase. However, in view of the absence of bone lesions it would seem unreasonable, in the present state of knowledge, to include this patient with the others.

DENTAL ASPECTS

The principal dental abnormality in hypophosphatasia is premature loss of primary teeth. The manifestation occurred in three-quarters of the children in group II and occasionally constituted the chief presenting feature (Cases 17 and 19). In Group I early loss of teeth was observed in only one case, but this was probably because few infants survived beyond the age of one year.

There appear to be two causes for premature exfoliation of the primary teeth in hypophosphatasia: (1) inadequate growth of alveolar bone [*36*], and (2) incomplete formation and early resorption of the roots of the teeth [*12*]. Other dental findings which have been reported include dental hypoplasia [*18*], enlarged pulp spaces [*7*], irregular calcification [*13*] and severe caries [*21,35*]. On the other hand, no radiologic or histologic abnormalities were observed in the primary teeth of our three patients despite the presence in each of lesions in the alveolar bone (Cases 9, 19 and 20). In the latter patients it is apparent that the metabolic derangement which resulted in an obvious defect in calcification of bone produced no demonstrable abnormality in calcification of the teeth. This anomaly suggests a difference in the genetic control of the cellular processes concerned with matrix formation or calcification in these two tissues. It deserves further investigation.

Little information has been recorded concerning the permanent dentition. The teeth appeared normal clinically and radiographically in Case 20. Dent reported severe caries in Case 32.

HISTOLOGY

Bone. A number of investigators have studied the histologic changes in hypophosphatasia, both

in the severely affected infants [*2,8,13,15,16,18*] and during the less acute phase [*5,10*]. In keeping with the radiographic changes the histologic defects were most marked at the growing regions of the bones. Both membrane and cartilage bone were involved.

It has been stressed that the lesions of hypophosphatasia are morphologically indistinguishable from those of simple rickets [*13,37*] unless special stains are performed to demonstrate phosphatase. In the epiphyseal plate the cartilage and the resting and proliferating cells near the epiphysis appeared normal. However, the hypertrophic chondrocytes close to the metaphysis had lost their normal columnar arrangement.

At the junction between cartilage and bone only occasional segments of the zone of provisional calcification could be distinguished. In this region tongues of apparently degenerating uncalcified cartilage projected toward the diaphysis, completely disrupting the normal architecture in this region [*2,13,16,18*]. In the metaphyseal regions islands of uncalcified cartilage were enveloped by uncalcified bone, and excessive amounts of uncalcified osteoid extended in a disorderly arrangement for several millimeters into the diaphysis. In the latter region the structure of the tissue approached that of relatively normal cortical bone, although several months after birth the shafts were abnormally small in diameter and cores of immature bone could still be seen surrounded by lamellar bone of mature type [*10,16*]. There was a considerable amount of irregularly calcified sub-periosteal osteoid which accounted for the radiographic changes seen in the shafts [*2,8,13,16*].

A number of special biophysical technics were employed by Engfeldt and Zetterström [*8*] in a study of undecalcified slices of affected bone. There was evidence of delay in the normal maturation of bone; linear growth and remodelling were defective; and there was a disturbance in the collagen structure and in the mineralization of the Haversian systems. *In vitro* uptake of Ca^{45} was reduced but mineral salts were deposited as hydroxyapatite crystals of normal form.

Similar defects in the growth and mineralization of the membrane bones have been described by several authors [*2,10,13,18*]. The widened "sutures" seen in radiographs of the skull were shown to consist of uncalcified osteoid tissue. Indeed, when the high incidence of craniostenosis is considered it may be of significance that the histological picture of a cranial suture has never been reported in this condition.

There are two fundamental matters upon which there is considerable disagreement. The first concerns the status of the osteoblasts and osteoclasts. Some authors [*8,10,15*] reported a paucity of both cell types at the costochondral junction and in trabecular bone. On the other hand, Park [*37*] believed that the osteoblasts were morphologically and numerically normal at the costochondral junction (Case 25).

The second point of contention concerns the morphologic appearance of the osteoid tissue. Some authors [*16,37*] have considered it normal whereas others [*8,10*] have suggested that the material appeared fibrous or hyalinized.

In essence, the histologic lesion in hypophosphatasia is characterized by two abnormalities, (1) a disturbance in the normal calcification process of cartilage and bone, and (2) excessive production and disordered arrangement of pre-osseous cartilage and of osteoid. If, as has been suggested, the osteoid is also qualitatively altered, then either abnormality theoretically could be the result of the other. It is not yet known which disturbance is primary in hypophosphatasia.

Kidneys. Autopsies have been reported in six of the ten fatal cases and in each, lesions were present in the kidneys. Deposits of calcium salts were observed in the renal tubular epithelium and in the surrounding interstitial tissues of five patients (Cases 2, 3, 4, 5 and 8) and calcium casts were sometimes present. In no case was nephrocalcinosis of sufficient degree to be visible radiographically. One is strongly inclined to attribute the nephrocalcinosis to hypercalcemia (*vide infra*) despite the reported absence of the latter feature in one case (Case 2).

In Case 1 no calcium deposits could be found in the kidneys. However, the collecting tubules were distended with casts and there was some necrosis of tubular epithelium.

It is not possible to assess the clinical importance of these lesions. Renal insufficiency, when present, was not great and it does not seem likely that the renal lesions were the primary cause of death in any of the children. Nevertheless, the potential danger of progressive renal damage in acutely affected infants with persistent hypercalcemia should not be underestimated.

Parathyroid Glands. A search was made for the parathyroid glands in each of the fatal cases cited. In three patients no glands could be located; in the others (Cases 4, 5 and 8) the parathyroid glands showed no significant lesions. Present evidence suggests that the parathyroid

glands do not play a primary role in the pathogenesis of hypophosphatasia.

Other Tissues. The adrenal glands were considered normal in four cases (Cases 1, 4, 5 and 8). The liver, thyroid, gonads and pancreas have shown no abnormalities. There have been no significant histologic changes reported in the pituitary.

CHEMICAL DETERMINATIONS

Serum Alkaline Phosphatase Activity. A marked reduction in the serum alkaline phosphatase activity is one of the cardinal features of hypophosphatasia. Values are not available for some of the patients but they have been recorded in twenty-five cases considered in the present review. (Table I.) The measurements have been made by a variety of methods, each with its specific substrate and conditions of reaction. Since the validity of the various conversion ratios is open to question, I have preferred to compare the figures obtained in a given case with the lowest value accepted as normal for the age of the patient and for the method employed.

Taking the group as a whole, the serum alkaline phosphatase activity in hypophosphatasia was reduced to approximately 25 per cent of the lower limit of normal, the mean levels varying widely from patient to patient, between almost no activity and 40 per cent of normal activity. There is no clear-cut relation between the degree of serum alkaline phosphatase depression and the severity of the clinical manifestations.

In following the alkaline phosphatase values of individual patients over a period of time, some showed remarkable constancy (Cases 1, 8 and 9). In others (Cases 10, 11, 15 and 25) a considerable variation occurred, without evident relation to the progress of the disease. Certain methods for determination of alkaline phosphatase are inaccurate at low enzyme concentrations, and it is possible that some of the variations noted are attributable to technical error.

Tissue Alkaline Phosphatase Activity. Several investigators have studied the alkaline phosphatase activity of various tissues in hypophosphatasia. Their findings regarding bone phosphatase have been [*2,7,8,10,13,15,16*] consistent and conclusive. They have demonstrated that the activity was markedly reduced in the metaphysis, the periosteum and the region of the costochondral junction during the active phase of the disease. For example, McCance et al. [*13*] found that the phosphatase activity at the costochondral junction of their patient was reduced to approximately 1 per cent of the amount present in normal infants, and the reductions recorded by other investigators, although not so marked, were definite nevertheless. To date, no tissue phosphatase studies have been carried out in bone from patients in whom optimal healing had already taken place.

Rathbun's discovery that phosphatase activity was reduced in bone, kidney and intestinal mucosa suggested the presence of a "generalized hypophosphatasia." His original concept has been confirmed by most subsequent studies. McCance et al. [*13*], employing different ester phosphate substrates, demonstrated an appreciable reduction in the phosphatase activity of liver, kidney and intestine. Engfeldt et al. [*8*] found the activity reduced to about 30 per cent in kidney, and Sobel et al. [*7*] noted a similar reduction in liver phosphatase activity.

To date, the only contradiction to the hypothesis of a generalized tissue phosphatase depression has been furnished by Scaglione and Lucey [*15*]. Their preliminary report indicated that the phosphatase level in the liver and the duodenal juice was normal in their patient. In view of the difficulties inherent in the measurement of tissue alkaline phosphatase activity [*38*], judgment upon this important contradictory finding should possibly be reserved until their data have been reported in detail.

Total Serum Calcium. Reference to Table I indicates that ten of the patients in group I had elevated serum calcium levels and that the value was normal or undetermined in six. However, when several determinations were carried out in a given patient wide variations usually occurred in the individual readings, and it is apparent that most of the hypercalcemic patients had normal serum calcium concentrations on occasion. The latter observation emphasizes the necessity of carrying out a number of determinations before eliminating the possibility of hypercalcemia in any given case. It is apparent that further study might well have uncovered additional cases of hypercalcemia among the less completely investigated infants in this group. Certainly, hypercalcemia is a common if not invariable occurrence in infants who are seriously affected. The degree of hypercalcemia was slight in some cases but in several, levels above 14.0 mg./100 ml. were recorded.

Hypercalcemia was a much less common finding in patients in group II, and when present was less severe. Five children were found to have elevated levels on occasion; the serum calcium values were normal or not reported for the remaining ten. Hypercalcemia was not observed in any of the adult cases (group III).

The cause of the hypercalcemia cannot be stated with finality. Since calcium balances have been invariably positive [*2,4,10,11,13*], since bone resorption would appear to be inhibited [*8*] and since there is no histologic or biochemical evidence of hyperparathyroidism, there is no indication that the excessive circulating calcium is of skeletal origin. Urinary calcium output was elevated in hypercalcemic patients (Cases 1, 10 and 21) and normal in normocalcemic patients (Cases 3, 25 and 35); therefore renal mechanisms are unlikely to be involved. One must conclude [*8,13*] that, for some unexplained reason, intestinal absorption of calcium takes place at a rate which exceeds the patients' ability to deposit the material in bone. On the basis of this conclusion it would seem logical to restrict calcium intake during the hypercalcemic phase of this disease.

Serum Inorganic Phosphorus. In general the serum inorganic phosphorus levels were normal. Individual determinations occasionally fell below or above the normal range for children but there were no consistent abnormalities. Terminal hyperphosphatemia was noted in a few cases.

Renal Function. As shown in Table I evidence of renal damage was present in ten of the infants in group I. In six the lesions were demonstrable at autopsy, and in four other patients (Cases 9, 10, 11 and 15) the presence of an elevated blood urea level and the demonstration of proteinuria and diminished concentrating power provided evidence of mild renal insufficiency. Elevation of the blood pressure was reported occasionally. No reference was made to renal function or urine in six cases.

In group II moderate renal insufficiency was present in only two cases. There was no nitrogen retention in eight other cases (Cases 17, 19, 20, 21, 25, 28, 30 and 31) and the urine was normal. No reference was made to renal function or urine in five cases.

Renal function was considered to be normal in two of the adult cases (Cases 32 and 35).

Other Determinations. A great many other determinations have been carried out on blood and urine but none have been significantly abnormal. The acid phosphatase, serum electrolytes, serum proteins, protein-bound iodine, Wassermann reaction and tests of adrenal cortical function have been normal. With the exception of the atypical case of Klein et al. both oral and intravenous glucose tolerance tests have been normal. On one occasion in Case 29 the fasting blood sugar was 30 mg. per 100 ml. Moderate anemia was present in some cases. When one considers the importance of phosphatases in biologic reactions it is surprising that evidences of metabolic disturbance have not been observed in other systems.

THE EXCRETION OF PHOSPHORYLETHANOLAMINE IN THE URINE

In the autumn of 1954 two independent groups discovered almost simultaneously the presence of a hitherto unreported ninhydrin-positive substance in the urine of infants with hypophosphatasia. Preliminary reports were made of these findings early in 1955 by McCance et al. [*39*] and by our group in Toronto [*40*]. At that time the evidence, based upon paper chromatographic technics, suggested that the material might be phosphorylethanolamine (amino ethyl phosphoric ester). Further evidence concerning the identity of the substance was presented by our group [*41*] in a paper delivered before the combined meetings of the American, British and Canadian Paediatric Societies at Quebec City, June 1955. We reported the isolation by ion exchange chromatography of a minute amount of crystalline material which behaved in a manner similar to synthetic amino ethyl phosphoric ester on ion exchange and paper chromatograms, and we showed that the material contained an ester-phosphate linkage as indicated by the technic of Hanes and Isherwood [*42*]. The chromatographic and electrophoretic properties of the substance were further discussed by Dent [*43*] at approximately the same time.

Recently, Cusworth [*44*] has succeeded in isolating a larger amount of the material from the urine of an adult patient with hypophosphatasia and has identified the substance as "phosphoethanolamine" by classic chemical methods.

Although phosphorylethanolamine had not been observed previously in human urine, it soon became evident that excretion of this compound was not limited specifically to patients with hypophosphatasia. Since the original reports detectable concentrations of what was

presumably the same material have been demonstrated on occasion in the urine of children with celiac disease [*45*], scurvy [*46*] and hypothyroidism [*46*], all of which diseases tend to have an associated suppression of the serum alkaline phosphatase level. On the other hand, the current studies of Dent and his associates [*35*] have suggested that phosphorylethanolamine is not detectable in the urine of randomly selected normal adults by their chromatographic procedure (1.25 mg. total nitrogen loading). The experience of our group has been similar, except that we believe small amounts of urinary phosphorylethanolamine may be detectable occasionally in randomly selected normal adults whose serum phosphatase levels are at the lower limit of normal.

Since its discovery, phosphorylethanolamine has been observed in the urine of several other cases of hypophosphatasia [*10,13,22,35,43*], and recently McCance et al. [*13*] have recorded for the first time the occurrence of a material which has been identified tentatively as adenosine monophosphate.

The question arises whether or not phosphorylethanolamine is invariably present in the urine of patients with hypophosphatasia. There is as yet little published information on this subject. Dent and Cusworth [*43*] found it in increased amounts in the urine of each of six established cases, and Harris and Robson [*47*] have identified it in each of fourteen. On the other hand, we have found it in only seven of eight patients. In the eighth, a typical case (Case 29), we were unable to detect even traces of phosphorylethanolamine on paper chromatograms loaded with 1.25 mg. total nitrogen—an amount five times the normal aliquot. It is unfortunate that it has not been possible to obtain another sample of urine from this patient, the only known non-excretor, since in studies of our other patients considerable differences have been observed in the rates of phosphorylethanolamine excretion.

As an explanation for the occurrence of phosphorylethanolamine in the blood and urine of patients with hypophosphatasia, it is tempting to suggest [*39,40,43*] that the substance accumulates because there is insufficient phosphatase to hydrolyse the ester phosphate linkage. This theory would suggest, in effect, that phosphorylethanolamine is one of the long-sought naturally-occurring substrates of alkaline phosphatase. In consonance with this concept is the recent demonstration by McCance et al. [*13*] that alkaline phosphatases derived from various normal tissues possessed the ability to hydrolyse synthetic phosphorylethanolamine *in vitro*. On the other hand, it is possible to propose other theories to explain the occurrence of phosphorylethanolamine in biologic systems. The latter cannot be excluded until more is known concerning the conditions which influence the excretion of this newly discovered substance.

GENETICS*

Schneider and Corcoran [*3*] were the first to propose a genetic factor in the etiology of hypophosphatasia. The evidence that has since accumulated fully supports their hypothesis. In early studies carriers were identified among the parents and relatives by the demonstration of low or borderline serum alkaline phosphatase levels. More recently the presence of increased amounts of urinary phosphorylethanolamine has also been considered an indication of heterozygosity. Unfortunately, in many papers on hypophosphatasia details which are of importance in evaluating the genetic aspects have been omitted, and for this reason the value of any conclusions is considerably reduced.

Fortunately, however, interpretation of the sex ratio is not affected by such shortcomings; although more females (twenty-one) than males (fourteen) were affected with hypophosphatasia, out of a total of thirty-five probands and affected sibs this deviation from a 1:1 sex ratio is not statistically significant (χ^2 of 1.0, 1 d.f., p is 0.30).

When more than one member in a family is affected with an uncommon anomaly the possibility of inheritance is suggested. Further, when both parents are apparently normal, and when, on the average, one-quarter of the sibs of the proband are affected, recessive inheritance of the disease and heterozygosity in both parents can be suspected. The parents were mentioned in fifteen of the families included in the present review; these parents were all considered normal as far as the bones were concerned, although to date no tissue biopsies have been studied. In twelve of these families (Cases 1, 2, 9, 10, 12, 18, 20, 21, 25, 26, 29 and 30) the serum of twenty-

* The genetic data have been interpreted by Professor N. Ford Walker, Department of Zoology, University of Toronto, and Director, Department of Genetics, The Research Institute, The Hospital for Sick Children, Toronto, Canada.

one patients was tested for alkaline phosphatase activity. The levels were low or border-line in sixteen persons and normal in the remaining five. The sixteen persons are therefore considered to be heterozygous.

The urine of six of these parents (Cases 1, 2, 9 and 20) was tested for the presence of phosphorylethanolamine. It was positive in four and negative in two. However, three of the four had already been classified as carriers on the basis of the alkaline phosphatase determination. One more heterozygote was thereby recognized, giving a total of seventeen heterozygous parents out of twenty-one, or 81 per cent—a proportion which approaches the expected 100 per cent. The four who were not classified as heterozygotes were tested only for phosphatase activity. It should be pointed out, however, that we have no proof at the present time that chromatography of the urine offers a more effective test of heterozygosity than does the serum phosphatase level. Nor have we sufficient data to prove whether or not serum alkaline phosphatase and phosphorylethanolaminuria are under the control of a single gene. Evidence [*46,47*] suggests at present that there is usually a reciprocal relation between the serum alkaline phosphatase level and the excretion of phosphorylethanolamine. However, in forty-nine estimations on seventeen probands or relatives [*46*] there have been three apparent exceptions which we are unable to explain by the hypothesis stated earlier.

Since unaffected children may not always have been recorded, the attempt to calculate the proportion of affected sibs of probands is the most unsatisfactory aspect of the present genetic analysis. The data, however, show that nine of thirty-four (26.4 per cent) sibs of the probands were affected, a close approximation to the one-quarter expected if recessive autosomal inheritance is involved. Of the unaffected sibs who were tested nine were found to be heterozygous (sibs of Cases 1, 9, 10, 21, 26 and 35), four were normal (sibs of Cases 1, 12, 27, 35)—approximately the 2:1 ratio expected.

In one instance an affected offspring of second cousins has been reported [*58*], further supporting the hypothesis of recessive inheritance.

A survey of the data presently available suggests that hypophosphatasia has a genetic basis, and that it may be inherited through the action of an autosomal recessive gene. It is to be hoped that further study of the biochemical and genetic aspects mentioned in the foregoing section will be stimulated by the preliminary findings reported herein.

NATURAL COURSE AND PROGNOSIS

It was pointed out in a previous section that the earlier the onset of pathology, the more severe the lesions. This axiom also holds with regard to the prognosis. Reference to Table I indicates that if lesions developed before birth a fatal outcome was to be anticipated within a few months. Of the sixteen patients included in group I, ten are known to have died, and others may have died since their histories were reported. Only five patients have survived beyond one year of age.

When the multiplicity of defects which were present in these severely affected infants is considered it is not surprising that few survived. Hypercalcemia, nephrocalcinosis and, in some cases, thoracic instability must have contributed to the fatal outcome. In addition, the possibility exists that, in severely affected newborn infants, generalized hypophosphatasia may have given rise to unrecognized metabolic derangements which were incompatible with life.

Insufficient stress has been placed upon the possible early deleterious effects of the severe cranial lesions which are characteristic of this group of infants. In a previous section I have given my reasons for considering that the bulging anterior fontanelle, typical of patients in group I, is an indication of a disparity between rate of brain growth and increase in intracranial volume. It is my thesis that this form of craniostenosis might account for the unexplained convulsions, cyanotic spells and sudden death which have been observed in some cases. In many cases surgical correction of the cranial lesion is probably indicated at this early stage.

Until recently little was known concerning the course of hypophosphatasia in untreated patients who survived the first few months of life. It is now becoming apparent that the illness passes through a cycle in which, initially, the manifestations become more severe, then gradually commence to decrease. From a prognostic standpoint it is important, but also very difficult, to predict the time relations in this cycle. As a general rule, patients who presented with lesions early in life tended to pass through the cycle more rapidly than patients with the less severe forms of the disease. Patients in whom lesions had developed in early infancy were ob-

served to deteriorate during the first year of life. Those who survived usually began to improve sometime during the first or second years of life but wide variations were observed in the rate and extent of the healing process. For example, radiologic improvement in the patient studied by Anspach and Clifton [*20*] did not commence until the third year of life, after which time there was a gradual but incomplete deposition of calcium in the metaphyses. Scaglione and Lucey questioned whether or not their patient had yet reached the turning point at the age of four years.

In contrast, the patients reported by Schlesinger et al. [*10*] began to improve at about one year of age; fairly rapid although incomplete radiologic improvement took place during the subsequent twelve-month period. Concurrently, the wide defects in calcification between the bones of the skull gradually became mineralized and, as the latter process neared completion, radiographs indicated that a total cranial synostosis had resulted.

Anspach and Clifton attributed the progress of their patient to radiation of the parathyroid glands, and Schlesinger et al. attributed the improvement they had seen to the administration of large doses of vitamin D. Other experience would cast doubt upon the efficacy of these measures [*6,7,11,16,35,48*], and the author believes that the improvement observed in the foregoing patients was independent of the therapeutic measures employed.

The radiographic improvement observed by Currarino et al. [*29*] in an untreated, severely affected infant was even more dramatic. Skeletal healing commenced between eight and twelve months of age and proceeded rapidly at first, then more slowly. By the age of twenty months certain of the long bones had become normal in appearance and the others were much improved. This case illustrates the rapidity with which bone healing may occasionally take place in hypophosphatasia.

To date, no relapses have been reported once radiologic improvement has become established. Present evidence indicates that a corresponding spontaneous improvement has not been observed in the low serum levels of alkaline phosphatase [*10,16,43,50*] or in the phosphorylethanolaminuria [*16*], and rarely in the hypercalcemia [*10*]. As yet there is no information concerning the long term progress of such infants. The prognosis must remain guarded with respect to the effects of persistent hypercalcemia and untreated complete craniostenosis (Case 11).

Recently, observations have been made [*16,49,50*] upon the progress of four less severely affected children (group II: Cases 20, 22, 23 and 24). In each, gradual improvement in the skeletal lesions had occurred over a period of years but the bones had not become normal. A normal height was attained in two cases (Cases 23 and 24) but was much reduced in one (Case 20). Renal function was normal. Little or no improvement was observed in the serum alkaline phosphatase activity. As Dent has suggested [*43*], it is possible that in children who fall within this group increased bone fragility will develop in adult life.

Although the outlook for complete cure of hypophosphatasia seems remote, the life expectancy of the less severely affected children is probably normal.

THERAPY

In the past nine years many attempts have been made to influence the course of hypophosphatasia. For the most part they have been completely unsuccessful. Vitamin C [*7*], thyroid extract [*2,7*], testosterone [*2*], growth hormone [*7*], thiamin [*7*], oral citrate [*16*] and benemid® [*3*] have been administered to patients with hypophosphatasia without any significant effect upon either phosphatase activity or skeleton.

Several groups [*4,6,7,10,11,16*] have administered large doses of vitamin D to these patients, on the basis of those few features of hypophosphatasia which superficially resemble vitamin D deficiency rickets. Initially, the results of this treatment were thought to show promise [*10*]. However, considered in the light of more recent information regarding the natural course of untreated cases, the beneficial effects of vitamin D therapy have been unconvincing. Furthermore, severe hypercalcemia occurred rapidly on numerous occasions [*6,7,10,11,16*]. In my opinion there is no justification at the present time for trial of this precarious form of therapy.

In 1956 a preliminary report [*51*] was published of our experiences with the use of cortisone in a severe case of hypophosphatasia (Case 9). Within three weeks of commencing an oral dose of 37.5 mg. of cortisone per day we observed that mineralization had appeared in the long bones at the zones of provisional calcification. Within the next seven weeks considerable cal-

cification had taken place in the metaphyseal and subperiosteal regions and the bones were approaching the normal. We were aware that fairly rapid healing of the bone lesions had occasionally been observed in untreated cases of hypophosphatasia; however, there were certain features of our case which convinced us that the dramatic skeletal improvement had not been entirely fortuitous. For instance, within a few days of initiating therapy we observed a definite and sustained rise in the serum alkaline phosphatase activity to approximately twice the pretreatment value, and a marked fall in the serum calcium concentration, features which have not been reported in natural remissions. When the hormone was withheld for two months skeletal lesions again became apparent, and the alkaline phosphatase activity fell to pretreatment levels; subsequently the bones healed rapidly when cortisone therapy was reinstituted.

At present, the child has been receiving 37.5 mg. of cortisone daily for one and one-half years. She is healthy and is growing slowly. Although certain of the epiphyseal plates remain distorted the metaphyses throughout the body appear to be completely and normally calcified. The serum calcium concentration is normal. The serum alkaline phosphatase level is slightly higher than during the pretreatment period but remains considerably less than normal, and phosphorylethanolamine is still present in the urine.

In support of our general findings Klein [*52*] has recently observed clinical improvement and a reversible increase in the alkaline phosphatase activity of his atypical patient during a period of cortisone therapy. On the other hand, preliminary trials of cortisone therapy in two affected children [*23,53*] and two adults [*22,35*] have failed so far to duplicate our findings. It is our present belief that cortisone has been of specific benefit to our patient. However, further confirmation of our observations is required in order to establish the efficacy of the treatment we have proposed.

The theoretical indications for early craniectomy and for restriction of calcium intake have been discussed in earlier sections.

FUNDAMENTAL NATURE OF HYPOPHOSPHATASIA

As the clinical features of hypophosphatasia have gradually unfolded during the past decade, so also the concepts regarding pathogenesis have become more clearly defined. Early investigators were struck by the markedly reduced alkaline phosphatase activity in the serum, and this observation has been extensively explored. Examination of urine, saliva and feces [*7,11*] failed to demonstrate excessive losses of enzyme. Sobel et al. [*7*], recognizing that the measurement of phosphatase activity did not necessarily represent the absolute concentration of the enzyme protein, searched in vain for evidence of possible phosphatase inhibitors. Further, it was shown [*16*] that magnesium, a known activator of certain phosphatases, was present in the serum in normal concentration. The incubation of serum from affected children with that of normal or rachitic persons has repeatedly failed to demonstrate the presence of non-specific inhibitors [*7,13,16*].

These findings lead to the conclusion that the low serum phosphatase activity reflects a true decrease in enzyme concentration, a decrease which theoretically could be caused either by an increased destruction or, more probably, by a decreased production of this group of enzymes in the tissues. In consonance with this hypothesis a marked reduction in tissue phosphatase concentration has invariably been demonstrated in bone, kidney and intestinal mucosa, and usually also in liver.

The possibility exists that the serum in hypophosphatasia might be unable to provide bone with an environment which could support mineralization. This suggestion seems unlikely, since it has been demonstrated [*40*] that adequate calcification took place *in vitro* when the cartilage of rachitic rats was incubated in the serum of a severely affected patient (Case 9). Similar *in vitro* experiments have provided further evidence that the seat of the disturbance lies within the bone itself. In contrast to the situation which obtains in cases of vitamin D resistant rickets [*54*], it has been shown [*41*] that no new deposition of bone mineral occurred when a slice of tissue from the patient's costochondral junction was incubated in the serum of healthy children.

All experimental data obtained to date have implicated the tissues, and for clinical purposes, principally the skeletal tissues, as the site of the pathologic process in hypophosphatasia. The lesion can be localized further to the proliferating osteoblasts [*2,8,10,15,16*] and the hypertrophic chondrocytes, since there is abundant evidence [*55*] that these cells normally constitute the principal source of bone phosphatase. On the basis of this cellular concept, the observed reduction in the phosphatase concentration must be

considered a secondary phenomenon. There is no doubt that studies of the phosphatase system in hypophosphatasia may help to answer some of the questions regarding the physiologic role of this enzyme in the formation of bone matrix and its calcification. Of more fundamental importance, however, is elucidation of the factors which give rise to the impaired function of the proliferating osteoblasts and the hypertrophic chondrocytes and, considering the disease in all its aspects, of phosphatase-producing cells throughout the body.

Whether the genetically determined disturbance in cellular function is caused by some deficiency, or by some inhibitor—exogenous or endogenous—remains a matter for speculation. In this connection the recent experiment of Scaglione and Lucey [*15*] is very stimulating, but the interpretation is open to argument. A block of bone was removed at operation from the skull of a patient who had a relatively severe hypophosphatasia (Case 16). The tissue contained no measurable phosphatase activity. A small fragment of the bone was placed in a tissue culture medium. During incubation there was a proliferation of cells which possessed the characteristics of osteoblasts, and it was observed that the new cells contained abundant phosphatase. It may be concluded that the culture medium had provided a more suitable environment for the proliferating cells than did the host. However, it is unjustifiable in the present state of knowledge to employ, without reservation, conclusions based upon *in vitro* results to explain observations made in the intact organism.

In a previous section of this review I have set forth in summary the reasons for our belief that cortisone exerts a specific effect upon the bone lesions in hypophosphatasia. In conformity with my concept of the fundamental nature of hypophosphatasia we postulated at one time that the disease process was caused by a derangement in adrenal function, and we explained the action of cortisone on the basis of its adrenal suppressive action. However, subsequent studies have not confirmed our original postulate, and we have put forward two additional explanations for the beneficial action of cortisone in our case. In the first, we postulate a direct pharmacologic action of cortisone on the disease process: in the second, we postulate that the beneficial action is exerted primarily in blocking some unknown toxic factor which, unopposed, acts as an osteoblast inhibitor. Both suggestions are in conformity with the basic concept of the pathogenesis of the disease but both are speculations which we cannot support by specific evidence. It is perhaps of interest that certain investigators [*16,43*] have tentatively suggested, each for his own reasons, the possible existence of vitamin D hypersensitivity as a factor in the pathogenesis of hypophosphatasia. Although there are a number of important drawbacks to such an hypothesis it fits in some respects the known facts concerning the toxic actions of vitamin D, and the postulated antagonistic effect of cortisone [*56,57*] upon the action of this vitamin.

In considering the present knowledge of hypophosphatasia it is obvious that little factual information is available and that much of the foregoing discussion is hypothetical. Perhaps this situation is inevitable for a condition so recently discovered and so rare. There is strong evidence that hypophosphatasia is genetically determined. Further, it is reasonable to suggest that the bone lesions are the result of a disturbance in the cellular processes concerned with either the elaboration of organic matrix, or its calcification, or both. Since the osteoblast is considered to play a key role in the control of both matrix formation and mineral deposition, and since the enzyme it elaborates, phosphatase, has been variously implicated in both processes, further elucidation of the primary disturbance in hypophosphatasia may contribute materially to the understanding of the fundamental physiology of bone.

TERMINOLOGY

In earlier papers on hypophosphatasia there was an obvious lack of agreement concerning nomenclature, and several designations have been applied. "Osteodysmetamorphosis fetalis" was suggested by the Swedish investigators [*8*] but the name is unwieldy and does not seem appropriate for those patients whose lesions are of postnatal onset. The term "rickets" has been used frequently in the past, perhaps with some justification. However, from almost every standpoint hypophosphatasia differs from the usually accepted connotation of rickets, and to use the latter term is to confuse the whole concept of hypophosphatasia.

Although future findings may suggest a more appropriate designation, the original term

"hypophosphatasia" would seem for the present to be the most satisfactory.

REFERENCES

1. Garrod, A. E. Inborn errors of metabolism. *Lancet*, 2: 1, 73, 142, 214, 1908.
2. Rathbun, J. C. Hypophosphatasia, a new developmental anomaly. *J. Dis. Child.*, 75: 822, 1948.
3. Schneider, R. W. and Corcoran, A. C. Familial nephrogenic osteopathy due to excessive tubular reabsorption of inorganic phosphate: a new syndrome and a novel mode of relief. *J. Lab. & Clin. Med.*, 36: 985, 1950.
4. Macey, H. B. Multiple pseudofractures; report of case. *Proc. Staff. Meet., Mayo Clin.*, 15: 789, 1940.
5. Sobel, E. H., Clark, L. C. and Robinow, M. Report on a disorder of the skeleton resembling rickets, associated with loss of teeth and deficient alkaline phosphatase activity in a 2-year-old child. *J. Dis. Child.*, 83: 410, 1952.
6. Clausen, S. W. Discussion quoted by Sobel et al. [*5*]. *J. Dis. Child.*, 83: 411, 1952.
7. Sobel, E. H., Clark, L. C., Fox, R. P. and Robinow, M. Rickets deficiency of "alkaline" phosphatase activity and premature loss of teeth in childhood. *Pediatrics*, 11: 309, 1953.
8. Engfeldt, B. and Zetterström, R. Osteodysmetamorphosis fetalis. *J. Pediat.*, 45: 125, 1954.
9. Luder, J. and Schlesinger, B. E. Osteodystrophy due to congenital deficiency of phosphatase. *Proc. Roy. Soc. Med.*, 47: 541, 1954.
10. Schlesinger, B., Luder, J. and Bodian, M. Rickets with alkaline phosphatase deficiency: An osteoblastic dysplasia. *Arch. Dis. Child.*, 30: 265, 1955.
11. Felder, J. and Schreier, K. Hypophosphatasie. *Monatssch. Kinderh.*, 103: 437, 1955.
12. Illingworth, R. S. and Gardiner, J. H. Premature loss of deciduous teeth. *Arch. Dis. Child.*, 30: 449, 1955.
13. McCance, R. A., Fairweather, D. V. I., Barrett, A. M. and Morrison, A. B. Genetic, clinical, biochemical and pathological features of hypophosphatasia. *Quart. J. Med.*, 25: 523, 1956.
14. Dent, C. E. Discussion on surgical aspects of disordered calcium metabolism. *Proc. Roy. Soc. Med.*, 49: 715, 1956.
15. Scaglione, P. R. and Lucey, J. F. Further observations on hypophosphatasia. *J. Dis. Child.*, 92: 403, 1956.
16. Fraser, D., Laidlaw, J. C., Munn, J. D., Walker, N. F. and Yendt, E. R. To be published.
17. Hühne, T. and Schönfeld, H. Eine eigenartige Wachstumsstorung in Kindersalter. *Monatssch. Kinderh.*, 42: 267, 1929.
18. Chown, B. Renal rickets and dwarfism; a pituitary disease. *Brit. J. Surg.*, 23: 552, 1935–1936.
19. Kubatsch, H. Über eine seltene Knochenerkrankung. *Monatssch. Kinderh.*, 75: 253, 1938.
20. Anspach, W. E. and Clifton, W. M. Hyperparathyroidism in children. *J. Dis. Child.*, 58: 540, 1939.
21. Harpur, E. R. Personal communication, 1955.
22. Henneman, P. H. Personal communication, 1956.
23. Laxdal, O. E. Personal communication, 1956.
24. Klein, R., Laron, Z., Knapp, J. and Sissman, N. A patient with hypoglycemia, renal glycosuria, and low serum alkaline phosphatase. *J. Dis. Child.*, 92: 514, 1956.
25. Neuhauser, E. B. D. and Currarino, G. Hypophosphatasia. *Am. J. Roentgenol.*, 72: 875, 1954.
26. Leucutia, T. Hypophosphatasia. *Am. J. Roentgenol.*, 73: 485, 1955.
27. Lanman, J. T. Hypophosphatasia—a newly recognized disease. *J. Pediat.*, 47: 509, 1955.
28. Engfeldt, B. and Zetterström, R. Osteodysmetamorphosis foetalis. In: Ciba Foundation Symposium. Bone Structure and Metabolism, p. 258. London, 1956. J & A Churchill.
29. Currarino, G., Neuhauser, E. B. D., Reyersbach, G. C. and Sobel, E. H. Hypophosphatasia. *Am. J. Roentgenol.* (In press 1957.)
30. Hansen, A. Phosphatase activity in serum and tissues of osteogenesis imperfecta. *Proc. Soc. Exper. Biol. & Med.*, 31: 1023, 1933–1934.
31. Jansen, M. Über atypische Chondrodystrophie (Achondroplasie) und über eine noch nicht beschriebene angeborene Wachstumsstörung des Knochensystems: Metaphysäre Dysotosis. *Ztschr. f. Orthop. Chir.*, 61: 253, 1934.
32. Parenti, G. C. La anosteogenesi: una varieta della osteogenesi imperfetta. *Pathologica*, 28: 447, 1936.
33. Müller, G. M. and Sissons, H. A. Case of renal rickets simulating metaphysial dysostosis. *J. Bone & Joint Surg.*, 33B: 231, 1951.
34. Cameron, J. A. P., Young, W. B. and Sissons, H. A. Metaphysial dysostosis. *J. Bone & Joint Surg.*, 36B: 622, 1954.
35. Dent, C. E. Personal communication, 1957.
36. Nikiforuk, G. and Fraser, D. To be published.
37. Park, E. A. Discussion, quoted by Sobel et al. [*5*]. *Am. J. Dis. Child.*, 83: 411, 1952.
38. Woodard, H. Q. Quantitative studies of β glycerophosphatase activity in normal and neoplastic tissues. *Cancer*, 9: 352, 1956.
39. McCance, R. A., Morrison, A. B. and Dent, C. E. The excretion of phosphoethanolamine and hypophosphatasia. *Lancet*, 1: 131, 1955.
40. Fraser, D., Yendt, E. R. and Christie, F. H. E. Metabolic abnormalities in hypophosphatasia. *Lancet*, 1: 286, 1955.
41. Fraser, D. and Yendt, E. R. Metabolic abnormalities in hypophosphatasia. *J. Dis. Child.*, 90: 552, 1955.
42. Hanes, C. S. and Isherwood, F. A. Separation of the phosphoric esters on the filter paper chromatogram. *Nature*, 164: 1107, 1949.
43. Dent, C. E. Discussion quoted by Engfeldt and Zetterström [*28*]. Ciba Foundation Symposium, Bone Structure and Metabolism, p. 266. London, 1956. J & A Churchill.
44. Cusworth, D. C. Personal communication, 1956.
45. Fisher, O. D. and Neill, D. W. Excretion of ethanolamine phosphoric acid in coeliac disease. *Lancet*, 1: 334, 1955.
46. Fraser, D. Unpublished.
47. Harris, H. and Robson, E. B. Personal communication, 1956.
48. Albright, F. and Reifenstein, E. C. The Parathyroid Glands and Metabolic Bone Disease, p. 99. Baltimore, 1948. Williams & Wilkins.

49. Forbes, G. Discussion quoted by Scaglione and Lucey [*15*]. *J. Dis. Child.*, 92: 494, 1956.
50. Forbes, G. Personal communication, 1957.
51. Fraser, D. and Laidlaw, J. C. Treatment of hypophosphatasia with cortisone. *Lancet*, 1: 553, 1956.
52. Klein, R. Personal communication, 1956.
53. Schlesinger, B. Personal communication, 1957.
54. Fraser, D., Jaco, N. T., Yendt, E. R., Munn, J. D. and Liu, E. The induction of *in vitro* and *in vivo* calcification in bones of children suffering from vitamin D resistant rickets without recourse to large doses of vitamin D. *J. Dis. Child.*, 93: 84, 1957.
55. Bourne, G. H. The Biochemistry and Physiology of Bone, p. 258. New York, 1956. Academic Press.
56. Dent, C. E., Flynn, F. V. and Nabarro, J. D. N. Hypercalcaemia and impairment of renal function in generalised sarcoidosis. *Brit. M. J.*, 2: 808, 1953.
57. Henneman, P. H., Carrol, E. L. and Dempsey, E. F. The mechanism responsible for hypercalcuria in sarcoid. *J. Clin. Invest.*, 33: 941, 1954.
58. Ulstrom, R. Discussion quoted by Fraser and Yendt [*41*]. *J. Dis. Child.*, 90: 554, 1955.

Wilson's Disease*

An Inborn Error of Metabolism with Multiple Manifestations

A. G. Bearn, M.D.
New York, New York

During the last decade information has accumulated which has permitted Wilson's disease (hepatolenticular degeneration) to be classified as an inborn error of metabolism. However, it should be admitted at the outset that the nature of the inborn error in Wilson's disease is still not known with any degree of certainty, that the etiology of the disease is understood in only the broadest outline, and that present day treatment still leaves much to be desired. Nevertheless, much about the disease has been learned not only concerning its genetic and biochemical features but also its clinical manifestations. In this account an attempt will be made to indicate how an understanding of the biochemical abnormalities helps to explain the varied and seemingly disconnected symptomatology of patients with Wilson's disease.

HEREDITARY ASPECTS

In common with other inborn errors of metabolism, the genetic component of the disease seems to be the decisive factor in the expression of the disorder. For the disease to become manifest clinically it is probable that the abnormal gene must be present in the double dose. The heterozygote under ordinary circumstances appears phenotypically normal. It is a paradoxic truism that the formal genetics of rare diseases are usually easy to elucidate, and Wilson's disease is no exception. A familial incidence, a high consanguinity rate, and an absence of the disease in the parents of those afflicted suggests a recessive mode of inheritance quickly confirmed by the application of mathematical tests designed to investigate such a hypothesis [*1*].

Estimation of the gene frequency and the carrier rate of the abnormal gene for Wilson's disease is extremely difficult since no information is available upon which reliable estimates can be made. The a priori method of calculating gene frequency devised by Dahlberg requires information both on the consanguinity rate of the parents of those afflicted, and on persons stemming from the same ethnic and cultural background who are unaffected by the disease. The observed first cousin consanguinity rate in a recent series of sixteen cases collected in New York City was 0.375.† If Dahlberg's calculation is performed assuming a consanguinity rate of 0.01 (Julia Bell's estimate for the consanguinity rate for a general hospital population was only 0.006) the calculated gene frequency is 0.001 with a carrier rate of 0.002. A consideration of the number of cases diagnosed in New York City, relative to the population size, indicates that the true gene frequency in New York is higher; this finding suggests that these cases stem from genetic isolates in which the normal consanguinity rate is also increased [*1*].

All patients with overt Wilson's disease, and who are thus assumed to be homozygous, have a disturbance in copper metabolism the details of which will be discussed later. Numerous attempts to detect a biochemical lesion in the heterozygote have been unrewarding. This failure should be ascribed to a lack of sophistication in the design of the experiments rather than to the absence of any biochemical abnormality for, except under unusual circumstances, the presence of even one abnormal allele will cause some defect in normal metabolism. In one instance, a characteristic biochemical lesion has been observed in the absence of overt clinical disease. This occurred in a young woman who

† The total number of patients with Wilson's disease studied has now been extended to twenty-six. Recalculation of the first cousin consanguinity rate of the parents reveals an incidence of 0.308, a value slightly lower than that obtained from an analysis of the first eighteen patients studied.

* From the Rockefeller Institute for Medical Research, New York, New York.

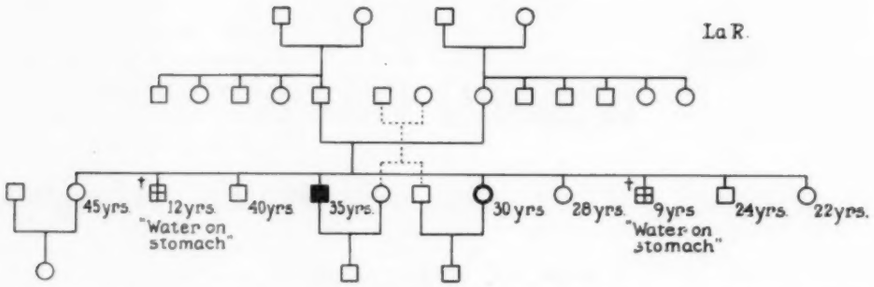


FIG. 1. Pedigree of patient LaR. Three siblings were affected, two of whom died in hepatocellular failure. The sibling, aged thirty, who is clinically normal, has a low serum copper, diminished serum ceruloplasmin and increased direct reacting copper. The urinary excretion of copper and amino acids is normal. No increase in her serum ceruloplasmin concentration occurred during pregnancy.

had three siblings with Wilson's disease. Follow-up studies will be required to determine whether or not clinical disease will eventually develop in this individual. (Fig. 1.)

CLINICAL FINDINGS

The essential clinical picture of hepatolenticular degeneration was clearly set forth in a monograph by Kinnier Wilson entitled, "Progressive Lenticular Degeneration: A familial disease associated with cirrhosis of the liver," published in 1912 [*2*]. The paper received immediate recognition but the very rarity of the disease precluded its systematic study by later workers. Although the disease is unquestionably rare, many cases have been misdiagnosed in the past. One of the chief reasons for the failure to recognize the disease has been a tendency for physicians to make the diagnosis only when the classic form of the disease is present. Detailed study has revealed many variations on the central theme of lenticular degeneration and cirrhosis of the liver. However, the crucial factor in our recognition of clinical variants of Wilson's disease has been the development of objective biochemical tests which are, with few exceptions, specific for the disease, and which serve as a reliable diagnostic arbiter.

The clinical manifestations of Wilson's disease usually arise during adolescence and the majority of cases occur between the ages of twelve and twenty. Most patients with Wilson's disease fall into two broad and overlapping clinical categories. In one, the symptoms are predominantly referable to the nervous system, in the other, the disease appears restricted to symptoms and signs of hepatic cirrhosis.

The neurologic form of the disease is most frequently seen. Symptoms of tremor, rigidity, dysarthria and dysphagia stem from disorganization of the lenticular region of the brain. In individual patients one symptom may predominate over the rest. When the disease becomes overt in young people it is more likely to be acute and progressive. Severe spastic rigidity dominates the clinical picture whereas tremor is only slight. In contrast, when the disease appears later in life it is more likely to be mild and only slowly progressive. Rigidity, although present, is usually minimal and tremor is more frequently the predominant symptom. Cirrhosis of the liver is almost invariably present in patients with Wilson's disease although it seldom becomes clinically overt. In most patients a slight increase in bromsulphalein retention is demonstrable but liver biopsy may be necessary to establish the diagnosis of hepatic cirrhosis with certainty.

Despite the brilliance of Wilson's clinical acumen one characteristic and pathognomonic feature of the disease curiously eluded him. In over 90 per cent of patients a brown or greyish green ring may be seen at the limbus of the cornea. The ring, frequently called a Kayser-Fleischer ring, may be partial or complete, unilateral or bilateral. Traditionally the ring is best seen with a slit lamp but in most patients this is an unnecessary refinement. The absence of a Kayser-Fleischer ring, while it does not exclude the presence of Wilson's disease, makes the diagnosis highly improbable.

In a few patients cirrhosis of the liver is the only clinical evidence of Wilson's disease. This group, although small in number, is important since these patients are most likely to remain undiagnosed. Hemorrhage from esophageal varices may arise early in the disease but ascites is usually delayed and is seldom severe. Although

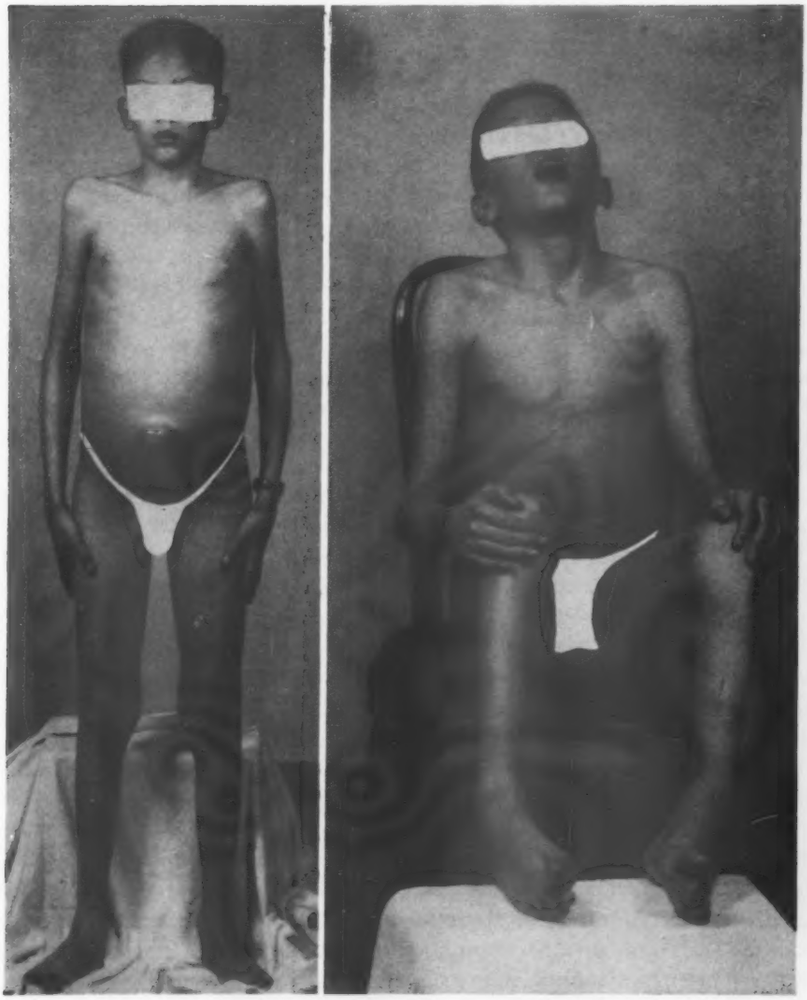


FIG. 2. The photograph on the left depicts a case of juvenile cirrhosis of unknown etiology in a boy of twelve. The photograph on the right illustrates the same patient one month later when gross neurologic symptoms of Wilson's disease had supervened.

examination of the central nervous system reveals no abnormal clinical signs, the presence of a Kayser-Fleischer ring will disclose the true diagnosis in the majority of patients. In most instances these patients, who are usually males, are diagnosed as having juvenile cirrhosis of undetermined etiology and die, either from hemorrhage or coma, without the correct diagnosis being reached. In other patients dramatic neurologic features may develop acutely and usher in the terminal phase of the disease. (Fig. 2.)

COPPER METABOLISM

In 1945 Glazebrook investigated a patient with Wilson's disease and analyzed the liver and brain for copper following the suggestive evidence in the old German literature [*3–5*] that copper might be an etiologic factor in its pathogenesis. The increased copper content of these organs found by Glazebrook [*6*] has been amply confirmed by a number of workers. The liver usually contains between five and twenty times as much copper as normal and the basal ganglia between five and ten times as much [*7,8*]. Recent work has demonstrated that the increased deposition of copper in the body of patients with Wilson's disease is not restricted to the liver and brain. An excessive quantity of copper is also found in the cerebral cortex, kidney and many other tissues [*8*]. The Kayser-Fleischer corneal ring probably also contains an increased quantity

of copper. In support of this opinion is the occasional occurrence of a "Sonnenblumenkatarakt" in patients with Wilson's disease [*9*]. Such cataracts are known to follow the intraocular localization of a foreign body containing copper [*10*].

Soon after the demonstration of an increased copper content of the tissues in Wilson's disease an increased urinary excretion of copper was reported [*11*] and rapidly confirmed. In contrast to the unanimity of opinion that the urinary excretion of copper was increased, the copper concentration of serum was reported to be either normal or elevated. Recent work has established that although normal values may sometimes occur, the serum copper is usually strikingly decreased [*12,13*].

Copper has long been known to be present in normal serum but little was known about the chemical nature of serum copper until 1948 when Holmberg and Laurell showed that about 95 per cent of the total serum copper was bound to a protein which behaved as a serum globulin and which they named ceruloplasmin [*14*]. Ceruloplasmin has a molecular weight of 151,000 and contains 8 atoms of copper tightly bound to each molecule of protein. The function of ceruloplasmin *in vivo* is unknown but, *in vitro*, ceruloplasmin has been shown to exhibit weak oxidase activity towards a variety of artificial substrates including paraphenylenediamine and benzidine [*15*] and, to a lesser extent, toward adrenaline and serotonin [*16*]. In conformity with the current one gene-one enzyme hypothesis it is tempting to think that ceruloplasmin may have an enzymatic function under physiologic conditions. The small amount of serum not bound to ceruloplasmin is loosely attached to serum albumin and can be considered to be copper in transport to various parts of the body.

In Wilson's disease the low serum copper is associated with a decreased quantity of ceruloplasmin [*1,17*]. In general, the depression of the ceruloplasmin is greater than would be predicted from the total serum copper concentration and in a few patients, although the serum copper is only moderately diminished, no serum ceruloplasmin can be detected. Cartwright and coworkers have demonstrated that the serum copper which is not bound to ceruloplasmin reacts directly with diethylthiocarbonate and that this fraction is increased in Wilson's disease [*13*]. Thus, although the serum ceruloplasmin is greatly decreased, the albumin bound copper (direct reacting copper) is increased in Wilson's disease.

RADIOACTIVE STUDIES

The use of radioactive copper in clarifying the defect in copper metabolism in Wilson's disease is limited by its short half-life (12.88 hours) and extensive experiments using this isotope are not possible. When radioactive copper is given, either orally or intravenously, to patients with Wilson's disease an increased urinary excretion of copper occurs compared to control subjects. Now, since the total body copper is greater than normal and since the urinary excretion of copper is increased, patients with Wilson's disease must be in positive copper balance. The difficulties inherent in the performance of a balance experiment involving trace elements can be overcome only partially by the use of Cu^{64}; nevertheless experiments performed in various laboratories have shown that an increased absorption of radioactive copper in Wilson's disease occurs [*18–20*]. Unfortunately, the experimental evidence is not as secure as in hemochromatosis in which increased iron absorbtion can be demonstrated convincingly by following the excretion of radioactive iron over many weeks.

Perhaps the most striking findings stemming from the use of Cu^{64} in this disease concern the metabolic fate of intravenously administered radioactive copper. In normal subjects a rapid decline in plasma radioactivity occurs, reaching its lowest level about four hours after administration. The plasma activity rises steadily thereafter and twenty-four hours later has still not reached a plateau. In contrast to normal subjects, patients with Wilson's disease show a slower fall in plasma radioactivity, but this decline continues and virtually no rise in plasma radioactivity is observed [*18,19*]. Electrophoretic separation of serum withdrawn at timed intervals after the administration of radioactive copper showed that initially the copper was bound to serum albumin but later became incorporated into serum ceruloplasmin. In patients with Wilson's disease the activity remained associated with serum albumin and little or no radioactivity became localized in the α_2-globulin region [*21*]. These experiments strongly suggest that serum ceruloplasmin is inadequately synthesized in patients with Wilson's disease. This concept has gained direct support from the work of Scheinberg who has calculated, on the basis of an

observed half-life of ceruloplasmin in patients with Wilson's disease of about four days, that if the decreased serum ceruloplasmin were due to increased destruction it would be necessary to postulate a normal half-life of ceruloplasmin of well over one hundred days. The magnitude of such a half-life is sufficiently greater than the reported half lives of other plasma proteins to make it intrinsically unlikely.

The variation in the level of serum ceruloplasmin in patients with Wilson's disease suggests that its synthesis is not depressed to the same degree in all patients. Thus the possibility arises that the serum ceruloplasmin could be raised, either by increasing its rate of synthesis or by delaying its normal metabolic breakdown. Attempts to delay the metabolic breakdown of the small quality of ceruloplasmin synthesized are beset by the difficulty that neither the mechanism nor the site of ceruloplasmin degradation are precisely established. Attempts to increase the serum ceruloplasmin by increasing the rate of synthesis of the protein have proved more fruitful.

EFFECT OF ESTROGEN OF SERUM CERULOPLASMIN

Serum ceruloplasmin, in common with other α_2 proteins, is frequently increased in disease states; elevated levels may occur in acute infections, myocardial infarction, thyrotoxicosis, schizophrenia and many other conditions [*23*]. A striking but unexplained rise in serum ceruloplasmin has long been observed during the latter months of pregnancy; after parturition the ceruloplasmin returns to normal. Recently Russ and Raymunt [*24*] have shown that oral estrogens given to normal subjects and patients suffering from a variety of diseases increase the serum ceruloplasmin levels. Thus it seemed possible that if a patient with Wilson's disease became pregnant, or if patients with Wilson's disease were given oral estrogens, an increase in the synthesis of serum ceruloplasmin might result. No patient with overt Wilson's disease thus far studied has become pregnant but pregnancy did occur in the asymptomatic sibling whose pedigree is illustrated in Figure 1. The pregnancy was uneventful and no rise in serum ceruloplasmin was observed. It is of interest, however, that this subject had a serum ceruloplasmin that was extremely depressed before pregnancy and hence might not have been expected to respond.

Seven patients with Wilson's disease have been given both natural and synthetic estrogens (estinyl and diethylstilbestrol) for periods up to five weeks. Determinations of serum copper, serum ceruloplasmin and direct reacting copper were made at weekly intervals. In some subjects the daily urinary excretion of copper was also determined. A summary of the maximum changes observed after four or five weeks of estrogen therapy is given in Table 1. In three patients (M. G., M. F. and P. C.) the serum copper rose to normal or supernormal values. In all instances this was accompanied by a rise in serum ceruloplasmin as calculated either from the difference between the direct reacting copper and the total serum copper, or by direct measurement of the oxidase activity of ceruloplasmin. The direct reacting copper showed no consistent change in these three patients. In three other patients (Y. R., M. A. and R. V.) no striking change in the serum copper, ceruloplasmin or direct reacting copper was observed. In one subject (L. A.) the ceruloplasmin increased slightly.

The three patients who responded the most had the highest initial ceruloplasmin level. In all instances, the ceruloplasmin level returned to pretreatment values when estrogen therapy was stopped. In one subject (M. G.) the serum copper and ceruloplasmin returned towards control

TABLE 1

EFFECT OF ESTROGENS* ON SERUM COPPER, SERUM CERULOPLASMIN AND DIRECT REACTING COPPER IN 7 PATIENTS WITH WILSON'S DISEASE

Patient	Therapy	Serum Copper (90–125 μg./100 ml.†)	Ceruloplasmin (3.0–4.0 μM./O_2/ml./hr.†)	Direct Reacting Copper (0–20 μg./100 ml.†)
Y. R.	Control	49	0.5	38
	Estrogens	40	0.3	30
L. A.	Control	75	0.7	48
	Estrogens	98	1.1	32
M. A.	Control	88	0.5	58
	Estrogens	70	0.9	43
M. G.	Control	84	1.5	..
	Estrogens	160	2.6	37
R. V.	Control	53	0.4	29
	Estrogens	50	0.4	33
M. F.	Control	96	1.1	24
	Estrogens	158	3.0	32
P. C.	Control	97	1.8	27
	Estrogens	267	5.4	30

* All patients received estinyl 1.0 mg./day except R. V. who received 0.5 mg./day and M. F. who received diethylstilbestrol 5.0 mg./day.

† Normal range.

values while he was still receiving estrogen therapy. In one patient (P. C.) the serum ceruloplasmin rose considerably above normal following estrogen therapy and coincidental with the rise in serum ceruloplasmin the serum bilirubin rose from 1.0 mg. per cent to 7.1 mg. per cent. The possibility that the rise in serum ceruloplasmin in this patient was in part due to decreased excretion by the liver must be considered. However, although an increase in the serum ceruloplasmin is a common finding in biliary cirrhosis in man [*12*], Cartwright and co-workers have reported that experimental ligation of the bile duct in dogs and pigs failed to increase the serum ceruloplasmin level [*25*]. The observed increase in serum bilirubin following estrogen administration was not altogether unexpected since a rise in bilirubin occurs in some cases of cirrhosis of the liver in young adults following similar therapy [*26*]. In the two patients studied (L. A. and M. F.) no change in the urinary excretion of copper was noted following administration of estrogen. Further work will be necessary to establish whether or not the urinary excretion of copper is elevated in patients who show a marked increase in serum ceruloplasmin after estrogen therapy.

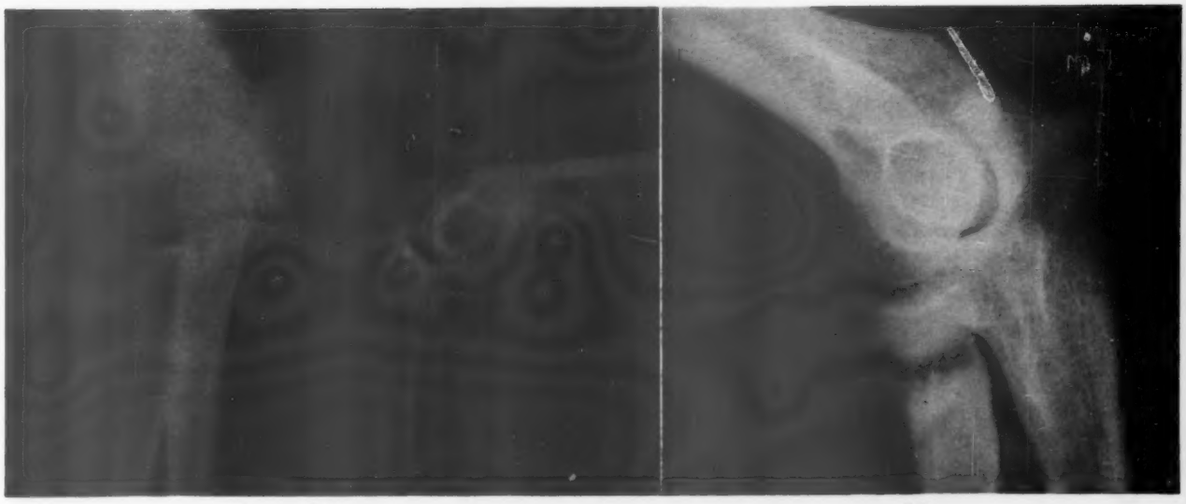
RENAL ABNORMALITIES

The casual observation by Dent in 1947 [*27*] of increased amino acid excretion in a patient with Wilson's disease with severe liver injury was followed by the important disclosure by Uzman and Denny-Brown that aminoaciduria was a fairly regular accompaniment of the disease, even though the plasma levels of amino acids were not elevated [*28–30*]. The finding of increased aminoaciduria in Wilson's disease coincided with the delineation of various hereditary aminoacidurias and, initially, it was tempting to place Wilson's disease into the same category, a view which in the light of further experimental data is no longer tenable. Although Wilson's disease is commonly associated with an increased amino acid excretion it is not an invariable concomitant of the disease. Some patients may exhibit classic Wilson's disease with normal amino acid excretion [*30*]. A detailed examination of the amino acids in the urine of patients with overt disease has shown that although, in general, the aminoaciduria involves a broad spectrum of amino acids there appears to be selective excretion of certain amino acids. The excretion of threonine and cystine may be increased up to twenty times normal. (The urinary cystine excretion may be as high as in cases of classic cystinuria.) Excretion of serine, glycine, asparagine, valine, tyrosine and lysine is increased between five and ten fold. Excretion of histidine, ornithine and phenylalanine is usually only slightly increased. Proline and citrulline, which are not found to any extent in normal urine, are excreted in the urine of patients with Wilson's disease in significant amounts. Taurine and methylhistidine, amino acids ordinarily present in urine, appear to be excreted in amounts slightly less than normal in some patients with Wilson's disease [*30*]. The precise significance of the particular pattern of aminoaciduria in Wilson's disease is obscure. Conjugated amino acids also are excreted in increased amounts in Wilson's disease. The question of whether or not patients with Wilson's disease excrete specific peptides, in addition to other conjugated amino acids, is less easily answered. Uzman has described the presence of peptides with dicarboxylic acid residues in the urine of patients with Wilson's disease [*31*] but thus far isolation and complete identification of these peptides has not been reported.

Aminoaciduria has been noted in a family with presumed Wilson's disease* [*32*] but it should be emphasized that the occurrence of aminoaciduria in the absence of disturbances in copper metabolism has not yet been reported in asymptomatic siblings or in patients with overt disease.

The discovery of excessive aminoaciduria prompted the search for increased urinary excretion of other metabolites normally reabsorbed by the proximal renal tubules. Glycosuria may occur in Wilson's disease [*29*] and is associated, in some instances, with a considerable reduction in the glucose Tm [*33*]. The blood glucose levels are usually in the low normal range. The urinary excretion of phosphate is also increased in Wilson's disease and may be associated with a low plasma phosphate level. In nine patients in whom renal clearance studies were performed, C_{PO_4}/C_{in} was invariably increased, whereas $C_{\alpha NH_2N}/C_{in}$ was increased in only seven [*33*]. The increased urinary phosphate excretion and the lowered plasma phosphate level may be in part related to the old observation that, on

* Recent unpublished results obtained by O. Forssman and C. B. Laurell suggest that this family is not suffering from classic Wilson's disease.



3A 3B

FIG. 3. A, patient M. G. Radiographs of the left elbow showing arthritic changes, bone irregularities and sclerosis. Note bone fragmentation along anterior and medial aspects of the joint. B, patient R. G. Radiograph illustrating fracture head of the right radius showing no evidence of bony union.

occasion, spontaneous fractures and osteomalacia may arise in Wilson's disease [*29,34*].

A systematic radiographic study of the bones of nineteen patients with Wilson's disease has revealed significant abnormalities in thirteen [*35*]. Severe degenerative changes around joints, particularly the spine, knees and elbows, associated with narrowing of the joint space, was commonly seen, even in young persons. A curious fragmentation of the bone in which small fragments of bone were seen around the joints of the hands and wrists was often evident. Classic osteomalacia was rare, but fractures resembling Milkman's lesions were seen in two patients. (Fig. 3.) The etiology of the bone lesions is obscure but it seems probable that factors other than the disturbance of phosphate metabolism enter into their etiology.

Increased urinary urate excretion accompanied by a low serum urate is another common metabolic finding in Wilson's disease [*36,37*]. Studies using N^{15} have shown that the total uric acid pool is reduced, and that the turnover rate is doubled [*36*]. The defect in renal reabsorption of urate may be so great that the uricosuria can not be further increased by administration of benemid.® Estimations of Tm_{PAH} also indicate severe impairment in the capacity of the renal tubules to excrete this substance [*33*].

The renal lesion in Wilson's disease bears a striking resemblance to that seen in the Fanconi syndrome but is not confined to a disturbance of tubular function. The glomerular filtration rate is usually lower than normal, as is the effective renal plasma flow. The filtration fraction is frequently slightly increased. The urine pH is usually high and urinary loss of bicarbonate may be somewhat excessive. Proteinuria, unassociated with tubular casts, is a common finding [*33*].

THERAPY

For many years the attitude towards the treatment of Wilson's disease was that of therapeutic nihilism. Recently, opinions have changed and we are now in danger of the pendulum swinging too far toward unwarranted optimism. However, in recent years the introduction of rational therapy has achieved some measure of success. Rational therapy has two principal aims; (1) to eliminate from the body the excessive copper that is already present, and (2) to prevent its reaccumulation. Attempts have been made to reduce total body copper by increasing the urinary excretion of copper.

In 1948 Mandelbrote and co-workers showed that the intramuscular injection of BAL (2,3, dimercaptopropanol) resulted in increased urinary excretion of copper [*11*]. Following the suggestion of Cumings [*7*] that BAL "might be used in an attempt to reduce the copper content of the liver and brain in Wilson's disease" many workers have used this compound with varying success. In some instances the administration of BAL has resulted in considerable clinical im-

provement [*9*]; in others, the disease has progressed. In the last analysis, any beneficial effect of BAL (or any other chelating agent) will depend upon its ability to achieve and maintain a persistently negative copper balance [*38*].

From a review of the literature and our own

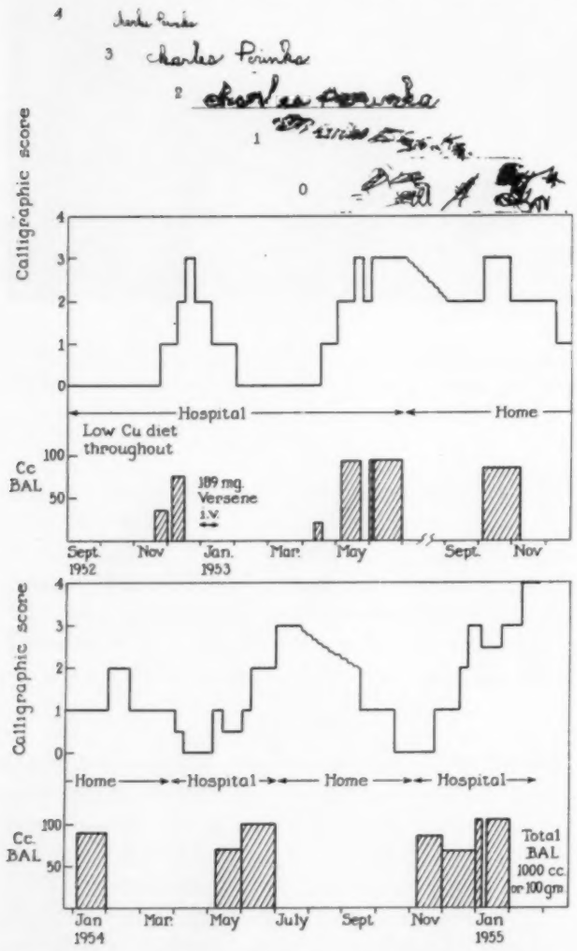


FIG. 4. Calligraphic chart illustrating the beneficial effect of BAL in one patient with Wilson's disease. The cross-hatched areas refer to the cumulative BAL dosage in any one course of treatment. The need for continuous therapy is apparent.

experience it is possible to divide patients with Wilson's disease into two groups which vary in their response to BAL. Improvement is seldom if ever observed in the group of patients characterized by severe progressive lenticular degeneration with accompanying widespread destructive lesions in the brain. The second group, who suffer from the pseudosclerotic variety of the disease, appear to respond more favorably. However, unequivocal evidence that any particular therapeutic regimen is beneficial in a disease characterized by a fluctuating course is difficult to obtain. In an effort to demonstrate that in some instances undoubted improvement can follow the administration of BAL, a patient of the pseudosclerotic type was admitted to the hospital and placed on a diet of fixed composition. After a suitable control period a therapeutic trial of BAL was instituted. In this patient improvement invariably followed administration of the drug and relapse promptly followed its withdrawal. (Fig. 4.)

It became increasingly apparent during the course of this experiment that if improvement is to be maintained continuous treatment is necessary. Unfortunately, the long continued administration of BAL by intramuscular injection is painful and the threat of local infection is always present. Moreover, in some patients toxic reactions to BAL arise which preclude its use [*38*]. Another chelating agent, versene (ethylenediamine tetra-acetic acid), has been used with some benefit but has the considerable practical disadvantage of requiring intravenous administration. Urinary copper excretion has been reported to be increased by intravenous infusion of amino acids [*39*] or more practically by a high protein diet [*12*].

Recently a considerable therapeutic advance has been achieved by the introduction of a powerful chelating agent that is effective by mouth. Penicillamine ($\beta\beta$-dimethylcysteine) has been shown by Walshe [*40*] to increase the urinary excretion of copper when given orally. Although penicillamine contains only one SH group whereas BAL contains two, penicillamine is more effective than BAL in increasing the urinary excretion of copper in patients with Wilson's disease. A comparison of the relative effectiveness of BAL and penicillamine is shown in Figure 5. Thus far supplies of the drug have been insufficient to answer definitely the question of whether or not penicillamine is more effective than BAL in achieving clinical improvement.

Since serum ceruloplasmin is deficient in Wilson's disease, logical therapy should include its replacement, either by continuous infusion of the purified protein, or better, by stimulating ceruloplasmin synthesis. Bickel has been unable to demonstrate any noticeable clinical improvement in three patients in whom continuous ceruloplasmin infusions resulted in restitution of the circulating ceruloplasmin to normal levels [*41*]. Similarly, patients in whom the ceruloplasmin level has been returned to normal

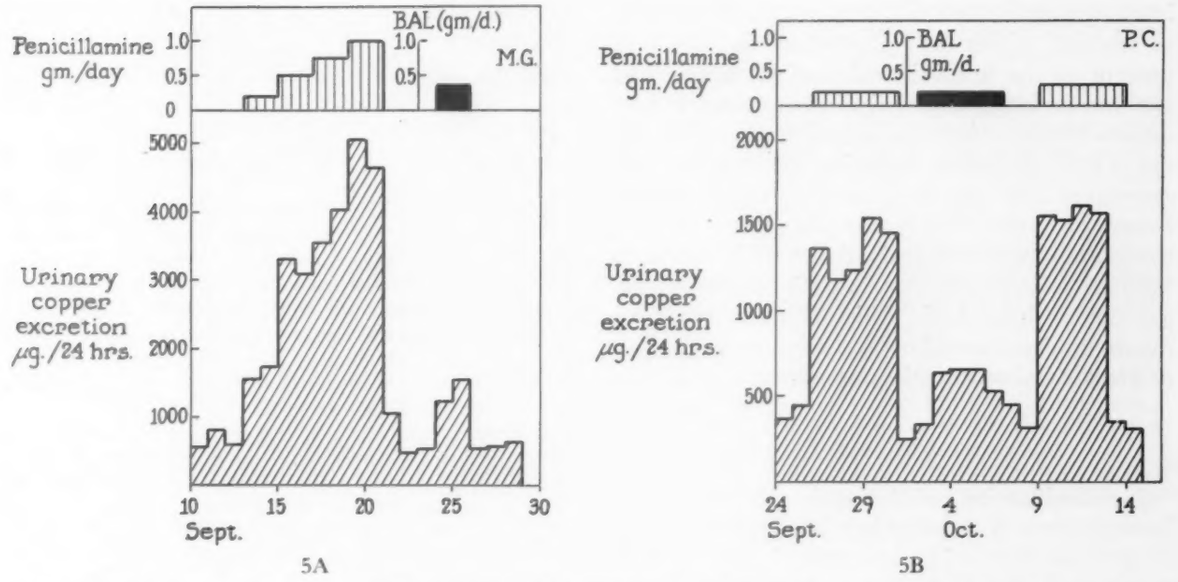


FIG. 5. A and B, a comparison of the relative effect of BAL and penicillamine on the urinary excretion of copper in two patients with Wilson's disease.

or supernormal values by the use of estrogens did not show any immediate clinical improvement [*42*]. It is clearly of great importance to know whether or not the return of the serum ceruloplasmin to normal levels decreases the absorption of copper from the intestinal tract. Thus far no conclusive evidence is available on this important subject. Whether or not clinical improvement will eventually occur remains to be determined.

TWO HYPOTHESES ON THE PATHOGENESIS OF THE DISEASE

Two different concepts of the pathogenesis of the disease are currently held. One hypothesis invokes an unusual affinity of certain tissue proteins for copper [*43*]. Consequently, excessive quantities of copper accumulate in the liver, brain and other tissues. The low ceruloplasmin is considered a secondary effect due to diversion of the available copper to tissues containing proteins with increased avidity for copper. Increased absorption of copper is in turn due to an increased copper binding capacity of the intestinal mucosa. The primary genetic defect is regarded by this school as "the formation of proteins with high copper affinity." The aminoaciduria and "peptiduria" are ascribed to incomplete tissue metabolism of these abnormal proteins, which leads to the formation and excretion of excess amino acids.

The second, and more currently favored hypothesis, is that the primary effect of the abnormal gene, when present in homozygous form, is to diminish the normal synthesis of serum ceruloplasmin and, possibly, other related proteins. Consequently, the copper attached to serum albumin cannot be utilized in the synthesis of ceruloplasmin. The copper bound to serum albumin persists at an elevated level, is excreted in the urine and is deposited in tissues where the affinity for the copper is greater than that of serum albumin. Accumulation of copper in the liver and brain leads either directly or indirectly to malfunction of these organs. Deposition of copper in the kidney may interfere with enzyme systems responsible for the transport of a variety of substances across the tubular epithelium and thus result in the renal lesion already described. It is readily admitted, however, that the present working hypothesis fails to explain all the experimental observations. It is not clear, for instance, why defective ceruloplasmin synthesis should cause increased absorption of copper from the intestinal tract or why feeding of a high copper diet to animals, although it results in increased tissue copper, fails to cause a condition in any way similar to Wilson's disease. (The possibility is not excluded that further experiments using other animal species will prove more successful.) The severity of the disease does not appear to be related to the depression of the ceruloplasmin

level, and restitution of the serum ceruloplasmin to normal, either by infusion of the purified protein or the administration of estrogens, has not thus far led to any clinical improvement. The improvement which may occasionally follow the use of BAL and other chelating agents is not well correlated with the magnitude of the increased copper excretion. The reason why, in some cases, the lesion is confined to the liver and in others to the brain likewise cannot be easily explained on the current hypothesis. This difference could be due to environmental causes, or more probably, to genetic influences still unassessed.

SUMMARY

1. Classification of Wilson's disease as an inborn error of metabolism is supported by a large amount of evidence concerning the hereditary and biochemical aspects of the disease.

2. The primary inherited defect appears to involve copper metabolism and is characterized by defective synthesis of serum ceruloplasmin, low serum copper and increased quantity of albumin-bound copper. An increased accumulation of copper in the liver, brain and other tissues occurs. The urinary excretion of copper is excessive.

3. The effect of estrogens on the serum ceruloplasmin concentration was studied in seven patients. In three patients, in whom the serum ceruloplasmin was the least depressed, administration of estrogens resulted in a rise in the ceruloplasmin levels to normal or greater than normal values; in one additional instance a slight rise was observed. In three patients no significant change in the serum ceruloplasmin level was demonstrable.

4. Accumulation of copper in the kidney in Wilson's disease results in a characteristic renal lesion, similar in many respects to that seen in the Fanconi syndrome. Glycosuria, aminoaciduria, phosphaturia and uricosuria may occur. In addition, an alteration in renal hemodynamics is usually demonstrable.

5. Radiographic examination of the bones and joints in nineteen patients with Wilson's disease revealed abnormal findings in thirteen. Severe osteoarthritis and bone fragmentation were common. More rarely, osteomalacia and spontaneous fractures also were seen.

6. The limitations of the various hypotheses advanced to explain the pathogenesis and the symptomatology of the disease are discussed.

Acknowledgment: I am greatly indebted to Dr Nathaniel Finby for his invaluable aid in the interpretation of the radiographs.

REFERENCES

1. Bearn, A. G. Genetic and biochemical aspects of Wilson's disease. *Am. J. Med.*, 15: 442, 1953.
2. Wilson, S. A. K. Progressive lenticular degeneration: a familial nervous disease associated with cirrhosis of the liver. *Brain*, 34: 295, 1912.
3. Rumpel, A. Über das Wesen und die Bedeutung der Leberveränderungen und der Pigmentierungen bei den damit verbundenen Fällen von Pseudosklerose, zugleich ein Beitrag zur Lehre von der Pseudosklerose (Westphal-Strümpell). *Deutsch. Ztschr. Nervenheilk.*, 49: 54, 1913.
4. Siemerling, E. and Oloff, H. Pseudosklerose (Westphal-Strümpell) mit Cornealring (Kayser-Fleischer) und doppelseitiger Scheinkatarakt, die nur bei seitlicher Beleuchtung sichtbar ist und die der nach Verletzung durch Kupfersplitter entstehenden Katarakt ähnlich ist. *Klin. Wchnschr.*, p. 1087, 1922.
5. Haurowitz, F. Über eine Anomalie des Kupferstoffwechsels. *Ztschr. physiol. Chem.*, 190: 72, 1930.
6. Glazebrook, A. J. Wilson's disease. *Edinburgh M. J.*, 52: 83, 1945.
7. Cumings, J. N. The copper and iron content of brain and liver in the normal and in hepato-lenticular degeneration. *Brain*, 71: 410, 1948.
8. Wintrobe, M. M., Cartwright, G. E., Hodges, R. E., Gubler, C. J., Mahoney, J. P., Daum, K. and Bean, W. B. Copper metabolism in Wilson's disease. *Tr. A. Am. Physicians*, 67: 232, 1954.
9. Denny-Brown, D. and Porter, H. The effect of BAL (2,3-dimercaptopropanol) in hepatolenticular degeneration (Wilson's disease). *New England J. Med.*, 245: 917, 1951.
10. Kjerrumgaard, E. Case of ocular chalcosis. *Acta ophthalm.*, 19: 261, 1941.
11. Mandelbrote, B. M., Stanier, M. W., Thompson, R. H. S. and Thurston, M. N. Studies on copper metabolism in demyelinating diseases of the central nervous system. *Brain*, 71: 212, 1948.
12. Bearn, A. G. and Kunkel, H. G. Abnormalities of copper metabolism in Wilson's disease and their relationship to the aminoaciduria. *J. Clin. Investigation*, 33: 400, 1954.
13. Cartwright, G. E., Hodges, R. E., Gubler, C. J., Mahoney, J. P., Daum, K., Wintrobe, M. M. and Bean, W. B. Studies on copper metabolism. XIII. Hepatolenticular degeneration. *J. Clin. Investigation*, 33: 1487, 1954.
14. Holmberg, C. G. and Laurell, C.-B. Investigations in serum copper. II. Isolation of the copper containing protein, and a description of some of its properties. *Acta chem. Scandinav.*, 2: 550, 1948.
15. Holmberg, C. G. and Laurell, C.-B. Oxidase reactions in human plasma caused by coeruloplasmin. *Scandinav. J. Clin. & Lab. Investigation*, 3: 103, 1951.
16. Abood, L. G. Communication to the Meeting of the Brain Research Foundation, Chicago, 1956.
17. Scheinberg, I. H. and Gitlin, D. Deficiency of ceruloplasmin in patients with hepatolenticular

degeneration (Wilson's disease). *Science*, 116: 484, 1952.

18. Bush, J. A., Mahoney, J. P., Markowitz, H., Gubler, C. J., Cartwright, G. E. and Wintrobe, M. M. Studies on copper metabolism. xvi. Radioactive copper studies in normal subjects and in patients with hepatolenticular degeneration. *J. Clin. Investigation*, 34: 1766, 1955.
19. Bearn, A. G. and Kunkel, H. G. Metabolic studies in Wilson's disease using Cu^{64}. *J. Lab. & Clin. Med.*, 45: 623, 1955.
20. Matthews, W. B. The absorption and excretion of radiocopper in hepato-lenticular degeneration (Wilson's disease). *J. Neurol., Neurosurg. & Psychiat.*, 17: 242, 1954.
21. Bearn, A. G. and Kunkel, H. G. Localization of Cu^{64} in serum fractions following oral administration: an alteration in Wilson's disease. *Proc. Soc. Exper. Biol & Med.*, 85: 44, 1954.
22. Scheinberg, I. H., Dubin, D. T. and Harris, R. S. The survival of normal ceruloplasmin in patients with hepatolenticular degeneration (Wilson's disease). *J. Clin. Investigation*, 34: 961, 1955.
23. Cartwright, G. E. Copper metabolism in human subjects. In: Copper Metabolism; A Symposium on Animal, Plant and Soil Relationships, p. 274. Edited by McElroy, W. D. and Glass, B. Baltimore, 1950. Johns Hopkins Press.
24. Russ, E. M. and Raymunt, J. Influence of estrogens on total serum copper and caeruloplasmin. *Proc. Soc. Exper. Biol. & Med.*, 92: 465, 1956.
25. Mahoney, J. P., Bush, J. A., Gubler, C. J., Moretz, W. H., Cartwright, G. E. and Wintrobe, M. M. Studies on copper metabolism. xv. The secretion of copper by animals. *J. Lab. & Clin. Med.*, 46: 702, 1955.
26. Bearn, A. G., Kunkel, H. G. and Slater, R. J. The problem of chronic liver disease in young women. *Am. J. Med.*, 21: 3, 1956.
27. Dent, C. E. Chromatography in the Study of Liver Disease. Tr. 6th Conference Liver Injury, Josiah Macey, Jr., Found., vol. 53, 1947.
28. Uzman, L. and Denny-Brown, D. Amino-aciduria in hepato-lenticular degeneration (Wilson's disease). *Am. J. M. Sc.*, 215: 599, 1948.
29. Cooper, A. M., Eckhardt, R. D., Faloon, W. W. and Davidson, C. S. Investigation of the amino-aciduria in Wilson's disease (hepatolenticular degeneration): demonstration of a defect in renal function. *J. Clin. Investigation*, 29: 265, 1950.
30. Stein, W. H., Bearn, A. G. and Moore, S. The amino acid content of the blood and urine in Wilson's disease. *J. Clin. Investigation*, 33: 410, 1954.
31. Uzman, L. L. On the relationship of urinary copper excretion to the aminoaciduria in Wilson's disease (hepatolenticular degeneration). *Am. J. M. Sc.*, 226: 645, 1953.
32. Uzman, L. L. and Hood, B. The familial nature of the amino-aciduria of Wilson's disease (hepatolenticular degeneration). *Am. J. M. Sc.*, 232: 392, 1952.
33. Bearn, A. G., Yü, T. F. and Gutman, A. B. Renal function in Wilson's disease. *J. Clin. Invest.* In press.
34. Warnock, C. G. Hepatolenticular degeneration (Wilson's disease); a report of five cases, with commentary. *Ulster M. J.*, 21: 155, 1952.
35. Finley, N. and Bearn, A. G. Radiographic abnormalities of the skeletal system in Wilson's disease (hepatolenticular degeneration). In preparation.
36. Bishop, C., Zimdahl, W. T. and Talbott, J. H. Uric acid in two patients with Wilson's disease (hepatolenticular degeneration). *Proc. Soc. Exper. Biol. & Med.*, 86: 440, 1954.
37. Mahoney, J. P., Sandberg, A. A., Gubler, C. J., Cartwright, G. E. and Wintrobe, M. M. Uric acid metabolism in hepatolenticular degeneration. *Proc. Soc. Exper. Biol. & Med.*, 88: 427, 1955.
38. Bearn, A. G. The place of BAL in the therapy of Wilson's disease. *Am. J. Med.*, 21: 134, 1956.
39. Matthews, W. B., Milne, M. D. and Bell, M. The metabolic disorder in hepato-lenticular degeneration. *Quart. J. Med.*, 21: 425, 1952.
40. Walshe, J. M. Penicillamine, a new oral therapy for Wilson's disease. *Am. J. Med.*, 21: 487, 1956.
41. Bickel, H., Schultze, H. E., Grüter, W. and Göllner, I. Versuche zur Coeruloplasminsubstitution bei der hepatocerebralen Degeneration (Wilsonshe Krankheit). *Klin. Wchnschr.*, 34: 961, 1956.
42. Bearn, A. G. Unpublished data.
43. Uzman, L. L., Iber, F. L. Chalmers, T. C. and Knowlton, M. The mechanism of copper deposition in the liver in hepatolenticular degeneration (Wilson's disease). *Am. J. M. Sc.*, 231: 511, 1956.

The Porphyrias as Inborn Errors of Metabolism

JAN WALDENSTROM, M.D.
Malmö-Lund, Sweden

THE importance of genetics for the study of metabolism and of metabolic disorders in man and animals has been convincingly demonstrated in the last decades. According to the writer's opinion the genetic classification is perhaps the only really reliable one that may be found in these conditions. It is now well known that in a family of hemophilia A or B (Christmas disease) all the members remain true to type, that is they are either A or B. The same has been demonstrated for the hemoglobinopathies, including the different types of hereditary methemoglobinemia. Dent's and Harris' extensive work on the different cystinurias have shown the same principle to hold true and my own earlier work on porphyria acuta seemed to demonstrate that this disease is fundamentally a unit apart from the other porphyrias and the symptomatic porphyrinurias. Later descriptions from a number of workers have obscured the picture by adding a number of so-called "mixed" porphyrias. It is evident that all these new facts must be collected and an attempt made to find out if new genetic disturbances can be defined. The influence of exogenous factors on the genotype must also be investigated.

Nomenclature. The disturbances of porphyrin metabolism associated with increased excretion of porphyrins in the urine and feces belong to one of two different groups: symptomatic or idiopathic. It has been known for approximately sixty years that certain poisons cause an increase in urinary porphyrins; such toxic porphyrinuria is one of the most important early and objective indications of lead poisoning. A large literature on porphyrin excretion in urine and feces in different diseases, the symptomatic porphyrinurias, has been published and the reader is referred to such reviews as those by Watson and Larson, Dobriner and Rhoads [*1,2*] for particulars.

The present paper deals with the *idiopathic* disturbances of porphyrin metabolism. These have been called *porphyrias* and should be distinguished from the *symptomatic porphyrinurias*. It must be remembered, however, that in a person who is porphyric a disease that causes porphyrinuria may also develop. The situation may then be quite complicated and I shall try to demonstrate that such a combination might explain some recent observations of so-called mixed porphyrias.

Günther [*3*] was the first to show that there were different types of porphyria cases, or what he called "Hämatoporphyrie." Since then several systems of classification have been adopted. The present author pointed out in 1937 [*4*] that congenital porphyria first revealed in childhood as severe sensitivity to light was completely different from the so-called acute porphyria, in which abdominal colic and sometimes paralyses and other neurologic symptoms dominate the picture. It was found that acute porphyria was not uncommon as a hereditary condition in Sweden, whereas porphyria congenita was not found in that country. The statement was therefore made that these two maladies should not be regarded as different types of porphyria but rather as completely separate diseases. The presence of porphobilinogen was regarded as characteristic of acute porphyria and its absence was noted in porphyria congenita. A third group of cases had been observed, in which the cutaneous symptoms developed late; these were called porphyria cutanea tarda. Subjects with cutaneous symptoms were never found among the numerous Swedish families with acute porphyria.

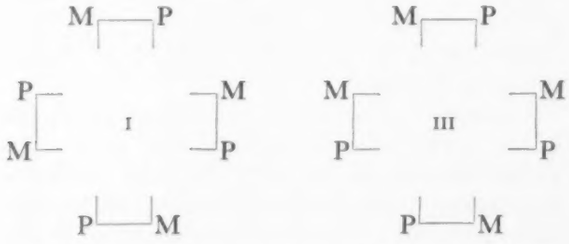
Later Watson and his group in Minneapolis, chiefly Schmid and Schwartz [*5*], arrived at the following classification which stresses the localization of the abnormal process. They call the first group photo-sensitive or erythropoietic, and the second, hepatic porphyria. In this latter group they recognize the following subdivisions: (a) intermittent acute, (b) porphyria cutanea tarda, and (c) a mixed type, a and b. In this

* From the Department of Medicine, Malmö General Hospital, University of Lund, Sweden.

paper I shall discuss these different systems and try to find a better classification, if possible.

Biochemical Considerations. Before entering upon the subject of heredity I must give a short survey of the chemical basis for the understanding of the porphyrias. Hematoporphyrin was found by Fischer to be a synthetic product not occurring in nature. The porphyrin in hemoglobin is a protoporphyrin with only two carboxyl groups. Normal feces also contains small amounts of a tetracarboxylated porphyrin which was called coproporphyrin by Fischer [*6*]. It is soluble in ether, as is the protoporphyrin. The presence of four more carboxyl groups makes this octacarboxylic acid insoluble in ether and most organic solvents. This substance was first isolated by Fischer [*7*] from the urine in a case of congenital porphyria and therefore called uroporphyrin. It is easily precipitated from the urine after slight acidification. Its spectroscopic characteristics are different from coproporphyrin but unequivocal identification requires purification and esterification, with melting point determinations of the esters. Copro- and uroporphyrins are most important for the diagnosis of the porphyrias.

It was also Fischer who first introduced the concept of a dualism among the porphyrins. This investigator found that the natural coproporphyrin occurs in two isomers. The β substituents of the four pyrroles are either methyl (M) or propionic (P). Their sequence may either be regularly alternating or irregular. The first Fischer called type I and he could show that the uroporphyrin in congenital porphyria belongs to this type. Protoporphyrin in heme is a type III porphyrin. Space does not allow a detailed discussion of the biological occurrence and importance of these isomers.



⌐¬ means an unsubstituted pyrrole.
M means methyl group.
P means propionic group.

In coproporphyrin there are four propionic acid substituents, in uroporphyrin the methyl groups are carboxylated. There is thus an alternation between acetic and propionic acids. Type III may be thought to arise when one pyrrole nucleus is rotated.

It was found by the present author [*4*] that a colorless pathologic substance also was regularly excreted in the urine in acute porphyria. This chromogen condensed to form uroporphyrin, which could be demonstrated spectroscopically. Its chief characteristic is a brilliant red color with the Ehrlich reagent. The Schwartz-Watson test is based upon this reaction [*8*]. The primary colorless chromogen was later called porphobilinogen (PBG) as it could couple with itself to form either a ring compound, uroporphyrin, or a red non-crystallizable chain compound, a pigment that we called porphobilin. Acidification and heating favored this process [*9*]. Porphobilinogen was first crystallized by Westall [*10*]. The recent brilliant chemical work of Rimington [*11*] and of Neuberger [*12*] and their groups in London, and of Shemin and colleagues [*13,14,15*] in New York, has shown that PBG is the biologic precursor of protoporphyrin and that the sequence must be a successive condensation of four molecules to uroporphyrin and subsequent decarboxylation from uro- [*8*] via copro- [*4*] to proto- [*2*] porphyrins or their derivatives.

Shemin has shown that PBG is formed through the condensation of two molecules of delta amino levulinic acid (ALA) [*16,17*]. It is now evident that a patient with acute porphyria may excrete not only porphobilinogen but also ALA [*18*]. Different disturbances in the whole series of intermediates between this simple amino acid and the highly complicated uroporphyrin may therefore account for the various biochemical and clinical manifestations of the different porphyrias.

The natural porphyrins are usually identified by their solubility in ether, their spectroscopic characteristics, and the crystal form and melting points of their esters. They all show a strong fluorescence in Wood's light. This accounts for the so-called photodynamic action that they share with other fluorescent pigments, for example, eosin.

CONGENITAL PORPHYRIA

Congenital porphyria is one of the rarest human diseases. Schmid et al. [*19,20*] recently made a critical study of all the published cases in the light of our present knowledge of other

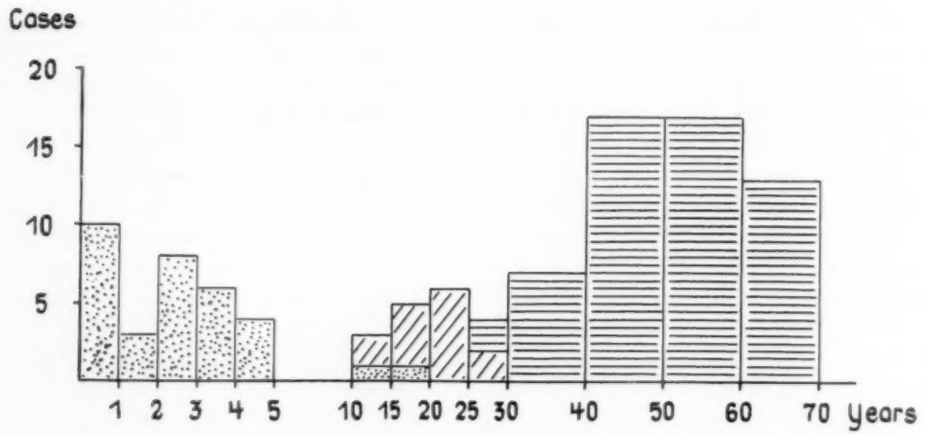


FIG. 1. Shows the age distribution of porphyria cases with cutaneous symptoms. Stippled areas indicate congenital porphyria. The age at onset of the two oldest cases is not definitely established. Diagonal lines indicate probable cases of protocoproporphyria. Horizontal lines indicate porphyria cutanea tarda symptomatica (from Brunsting, Tappeiner and Bolgert, and from some other authors. Some of the younger cases are regarded by Brunsting as mixed).

forms of porphyria. They found that the majority of cases in the literature did not conform to the rigid and logical criteria of photo-sensitive or erythropoietic porphyria and they accept only thirty-four instances as true congenital porphyria. (Fig. 1.) The others they regard as porphyria cutanea tarda. Nineteen were females, fifteen were males. The excretion of porphyrins in the urine is usually present from birth. The mothers note that the child's diapers are colored red by the urine. The urine contains chiefly uroporphyrin and some coproporphyrin, but never PBG; it is therefore red because of the presence of porphyrins. Porphyrins are present in the blood. Splenomegaly and anemia are common symptoms. Both the deciduous and permanent teeth may be colored red or brownish and fluoresce strongly in Wood's light. This is explained by the fact that uroporphyrin is precipitated in the bony substance. The skeleton is therefore brownish, fluoresces vividly in ultraviolet light and contains large amounts of uroporphyrin. The feces and bile contain coproporphyrin in large amounts.

These unfortunate patients suffer from severe light sensitivity. Ultraviolet light causes erythema, bullae and ulcers, depending on the degree of exposure. The face and hands may mimic leprosy, with severe mutilations of the finger tips, ears and nose. The heroic experiment of Meyer-Betz, who injected hematoporphyrin into himself and in whom severe cutaneous symptoms developed after light exposure, definitely proved the role of the fluorescent porphyrins in the disease. It is very difficult to provide sufficient protection against the actinic influences even of daylight and the patients are therefore better off if they can work at night and stay in the dark during the day. In some patients however, light sensitivity alone does not dominate the picture. The erythropoietic function also is markedly affected. This problem has been beautifully elucidated by Schmid, Schwartz and Sundberg [*5*]. These workers showed, with the aid of the fluorescence microscope and an extremely refined fluorescence microspectrograph, that some erythroblasts in the bone marrow contained large amounts of uroporphyrin. The severe anemia that may develop early in these patients was thought to arise, in part, from insufficient formation of protoporphyrin from uroporphyrin. The porphyria is "erythropoietic." A hemolytic component is obviously also at work, as some patients have improved considerably after splenectomy. I shall not discuss these clinical problems in detail.

It seems probable that congenital porphyria is an inborn error of metabolism, as was assumed by Garrod in 1923 [*21*], but we know surprisingly little about the heredity of this condition. The trait has never been observed in successive generations. It has usually been assumed that a rare recessive gene is responsible but the number of cousin marriages among the patients' parents noted in the publications is remarkably small [*22*] and occurrence among several siblings is also uncommon. (Table I.)

It is therefore not definitely proved that the disease is hereditary in man. A similar disturbance has been noted in pigs in Denmark [*29*] and in cattle in South Africa. Rimington started his important work on porphyrins in 1936 at Onderstepoort with a study of such animals. The bull from which a number of affected animals descended was crossed with one of his offspring. Neither of the parents showed any signs of disease but a male calf had typical porphyria congenita [*30*]. This definitely indicates that the affected animal was homozygous. It seems proved that bovine congenital porphyria is an inborn error of metabolism with recessive heredity and the analogy with the human disease would appear to be close.

TABLE I
CONGENITAL PORPHYRIA IN SIBLINGS

Reference	No. Affected
Anderson [*23*]	Two brothers affected, probably one sister
Sato and Takahashi [*24*]	Two sisters severely anemic, died before the age of two
Kitagawa, Matsuoka [*25,26*]	Brother and sister
Hernando (Corisa) [*27*]	Two sisters
Hernando (Medina) [*27*]	Three siblings
May et al. [*28*]	Two brothers, one sister, six healthy siblings

PORPHYRIA CUTANEA TARDA (PCT) SYMPTOMATICA

The original concept of porphyria cutanea tarda (PCT) as a definite entity requires revision. By definition, this type of porphyria was expected to show cutaneous symptoms late in life, in contradistinction to the congenital type with onset in early childhood. In this latter condition the cutaneous manifestations also were usually severe and disfiguring. The literature (Table II) as well as my own experience indicates that PCT is usually found in men more than 40 years of age. The reason for this male preponderance is that nearly all these patients were alcoholics. Even among the women this holds true. Signs of disturbed liver function are usually present. Liver cirrhosis of severe degree is not common and postmortem examinations are scarce. One patient that I have seen in consultation was later reported on by Paul, Engström and Engfeldt [*37*]. It was found that the skeleton was brownish and contained large amounts of uroporphyrin. The liver showed severe cirrhosis.

I shall not enter upon a discussion of the clinical picture in PCT symptomatica. The photosensitiveness is not as marked as in porphyria congenita but it is typical that the lesions occur on exposed skin areas (face, hands, arms). The mechanical factor is obviously of great importance in the production of blisters that may later ulcerate and lead to scar formation. Intense pigmentation is characteristic, as is also the occurrence of hypertrichosis on the face. The reader is referred to the excellent reviews by Brunsting [*32*] and by Tappeiner [*36*].

TABLE II
SEX AND AGE DISTRIBUTION IN PORPHYRIA CUTANEA TARDA SYMPTOMATICA

Reference	Country	Men	Women	Age (yr.)	
				Minimum	Maximum
Szodoray and Sümagi [*31*]	Hungary	10	2	26	64
Brunsting [*32*]	U. S. A.	26	8	34	65
Watson [*33*]	U. S. A.	19	4	..	...
Borda [*34*]	Argentina	3	3	35	56
Bolgert et al. [*35*]	France	7	2	35	68
Tappeiner [*36*]	Austria	11	1	32	66
		76	20		

There is, however, a small number of cases in which alcoholism cannot be suspected. These patients belong to two different age groups. The first is comprised of young persons usually in or immediately after puberty. The other is comprised of elderly persons of both sexes. The most striking instances in both groups were recently published by Tiong Hoo [*38*]. This author observed an 80 year old woman who had suffered from blisters during the preceding year. She had sixteen brothers and sisters, who had never had any similar complaints. Her five children were healthy also, and chemical examination of urine and feces gave normal findings. The diagnosis of porphyria cutanea tarda was made. It was also found, however, that she had a large palpable tumour in her abdomen. This was resected and was diagnosed as liver cell adenoma (weight 520 gm.). It looked benign histologically, showed strong fluorescence in Wood's light and contained considerable amounts of proto-, copro- and uroporphyrin but no porphobilinogen. Her urine before the operation had contained considerable amounts of uroporphyrin and smaller amounts

of coproporphyrin but no porphobilinogen. Her serum fluoresced strongly before the operation and this continued for about one month after the operation and then disappeared. Most interesting was the finding that the excretion of porphyrins in the urine also returned to normal values after the operation. At the same time the condition of her skin became normal. She has been re-examined by Dr. Hoo for one year and a half and has remained perfectly well. It is remarkable, however, that the content of proto- and coproporphyrin in the feces never returned to really normal values. This woman obviously suffered from a clinical picture that must be diagnosed as porphyria cutanea tarda. The definition of porphyria does not seem to apply to this case, however, as her porphyrin disturbance must have been caused by the liver cell adenoma and was therefore symptomatic and could be abolished by operation. Her family history, furthermore, was entirely negative. This is a unique observation of spurious porphyria in which the site of the metabolic defect was extirpated. Parallels might be drawn with chorionepitheliomas producing gonadotropin. Similar is a case of malignant argentaffinoma contained in an ovarian teratoma, in which the whole metabolic effect disappeared after extirpation of the tumour [*39*]. Another case that is probably similar has been published by Hamminga [*40*], curiously enough also from Holland. PCT developed in an old lady who had tumors in the liver. There was no histologic confirmation however.

PORPHYRIA CUTANEA TARDA HEREDITARIA (PROTOCOPROPORPHYRIA)

The other group comprising a number of youngish persons has not been clearly defined. The best description is by Tiong Hoo [*38*]. This author investigated a family with clinical symptoms typical of PCT. There were five affected females and four affected males. The only remarkable thing was the fact that blisters occurred on the skin at an early age (in two at twenty-three years, in three at sixteen, in one at seventeen, in one at twenty-two, in one at twenty-eight and in one at thirty). (Fig. 1.) It is evident from the data in the pedigree that both copro- and protoporphyrins were present in large quantities in the feces and that uroporphyrin was sometimes identified in the urine, consistently so in only three patients, and in much smaller amounts than coproporphyrin. PBG was always absent. The chief disturbance probably lies in the excretion of protoporphyrin in the bile, and also of a smaller amount of coproporphyrin by the same route. Wells and Rimington [*41*] have observed a brother and sister, aged twenty-five and nineteen when the symptoms began, who showed the same metabolic disorder. It should also be noted that the patient described by MacGregor, Nicholas and Rimington [*42*] was a boy who started to have his symptoms at the age of eleven. The girl observed by Discombe and Treip [*43*] was twenty years old, and a patient described by Gray and Rimington [*44*] was a boy of twenty-two. Calvert and Rimington's patient [*45*] started to have blisters at the age of twenty-four. At twenty-six he had a typical severe attack of acute porphyria with quadrilplegia. Only then was PBG found, in addition to uroporphyrin. These young persons always excreted large amounts of proto- and coproporphyrin in the feces during a remission. It was noted by Rimington, however, that during an attack the feces contained less porphyrins, whereas the urinary output increased considerably. This reciprocity in the excretory function of liver and kidney is, according to Rimington, the fundamental fact in this disease. These patients suffer from an inborn error of metabolism that might perhaps be called protocoproporphyria. As long as the liver is able to take care of the porphyrin and excrete it through the bile the patient suffers no harm and only careful chemical examination of the stools will reveal the presence of the malady. Rimington [*30*] has drawn an interesting parallel to "icterogenin" jaundice that occurs in sheep who have been grazing on Lippia rehmanni and have suffered secondary damage to the liver. Patients with this type of porphyria are usually icteric during the attack and the alkaline phosphatase values may be increased. Bilirubinuria may also be found. The case histories are presented in great detail, with an excellent discussion, and the reader is referred to the original papers for clinical information.

Hijmans van den Bergh and Grotepass [*46*] in Holland had earlier noted two cases in which there was porphyrinemia without porphyrinuria. Uroporphyrinuria was quite transitory but serum, bile and feces contained large amounts of proto- and coproporphyrin. One of these patients had cutaneous symptoms. The ages of these patients are not stated. Similar cases of

idiopathic coproporphyrinuria, as they have been called by Watson, have been described by Taylor et al. [*47*] and by Watson [*33*]. One of Watson's two patient's also had protoporphyrin in his feces; the other had only coproporphyrin. Porphobilinogen could never be found in these cases but it is interesting to note that MacGregor and Rimington's patient [*42*] had a number of attacks of severe colic and died in the last attack with mental confusion, paroxysmal hypertension and respiratory paralysis. During this last phase porphobilinogen was found in the urine. (See also [*45*]). This seems quite important for the discussion of a connection between porphobilinogen and nervous symptoms. To my mind this is not a *mixed* porphyria. The symptoms indicate breakdown of porphyrin metabolism generally, with excretion of precursors only during the attack.

Comments. To sum up our present knowledge of cutaneous porphyria it might be said that there are three or possibly four different types. A large number of cases occur in chronic alcoholics. This explains the general preponderance of males. Liver damage is probably the cause in these cases, as well as in the many observations of PCT in the South African Bantu population [*48*]. Another type, in which the disturbance also is symptomatic, is the patient with liver cell adenoma [*38*], and probably one of my own observations as well as the case reported by Hamminga [*40*]. These types are not true porphyrias. Chronic protocoproporphyria mainly in the bile and feces, probably inherited as a dominant trait, may be completely symptomless and therefore remain undetected or give rise to intermittent attacks with jaundice, bilirubinuria, porphyrinemia and light sensitivity probably caused by transient hepatic insufficiency. (Reciprocity theory of Rimington). This malady usually first becomes manifest about puberty. Only when porphyrin metabolism is severely disturbed may porphobilinogen appear in the urine and only in such cases have colic and paralysis been noted (as in acute porphyria). The sequence may be: (1) latent (feces) (2) cutaneous (urine) and, rarely, (3) the final appearance of PBG in the urine and symptoms of AIP. Idiopathic coproporphyrinuria probably belongs to this group but the family recently described by Berger and Goldberg [*49*] is more difficult to group among the porphyrias until more observations are available. No light sensitivity was found in these patients. Some other cases in the literature are also difficult to group.

In the so-called mixed or combined cases described by Watson, by Brunsting and above all by Dean and Barnes occurring in Caucasians in South Africa [*50*] the subjects possibly suffer from acute porphyria, with some superimposed, possibly toxic, influence. They will be discussed in the next section. The case published by Kuske [*51*] is of great interest but lacks analysis of the porphyrins and of the genealogy.

ACUTE PORPHYRIA

It has already been pointed out that the designation of the third large group of the porphyrias, namely acute porphyria, really is misleading. The metabolic defect obviously is very often inherited, that is, it must be present in some form at birth. The metabolic disturbance is therefore distinctly chronic, even if there are attacks of an acute type. The term "porphyria" should probably be dropped as the malady really is a profound basic disturbance in pyrrole metabolism. The name "pyrrolia" has been somewhat facetiously suggested by the present author! The best designation is probably the addition of intermittent to acute, as was first suggested by Watson. I shall therefore call this disease acute intermittent porphyria (AIP) in what follows.

The clinical picture is quite uniform in most cases, even if different types may be distinguished. The latent form is diagnostically important as most patients show some metabolic stigmata even in the periods between attacks. It is interesting to note that such stigmata have never been found in children who must be carriers of the trait when manifest AIP later develops. This is quite compatible with the fact that manifest AIP is practically unknown in young children. Very rare exceptions were published by Watson [*33*]. It is seen from the diagram (Fig. 2) constructed from our data on 219 manifest cases in Sweden that the age of onset of symptoms usually is some time after puberty. Rarely, however, symptoms appear for the first time much later in life. Only five patients had their first symptoms before fifteen years of age. There are 131 males and 190 females in the whole group; in our largest family the sex distribution is exactly the same, that is, fifty-four males (40 per cent) and eighty-three females (60 per cent).

Abdominal colic is not only the usual initial

symptom but also the most common sign of the disease. These colics are usually extremely painful and patients have told me that the pain they experience in the abdomen cannot be compared to other types of pain such as childbirth, lithiasis or a severe toothache. The attack

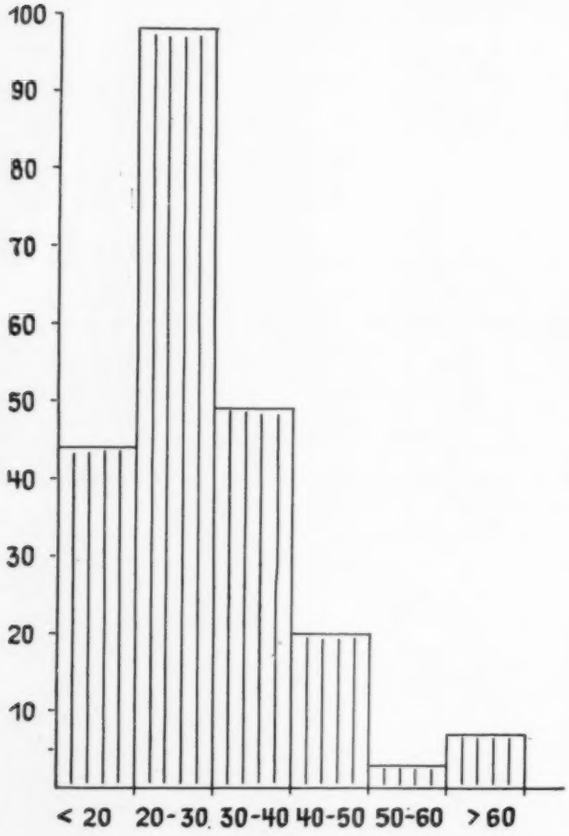
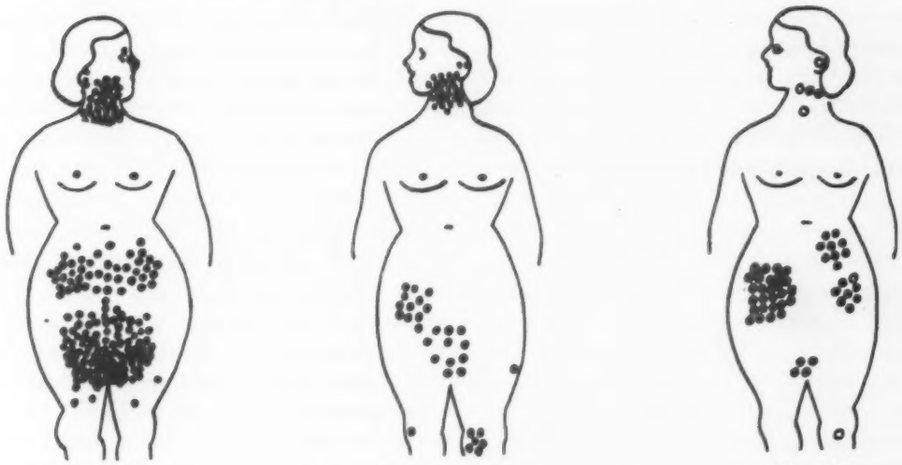


FIG. 2. Age at first manifestation of acute intermittent porphyria (AIP) in Sweden.

is a typical colic. The patient wishes to press something against his abdomen and this desire sometimes causes an erroneous interpretation of the condition as hysterical. Loud shrieks and a curious position in bed are in themselves rarely noted in other somatic abdominal diseases and the fact that psychic symptoms, often of a psychotic nature, may follow the abdominal complaints makes it understandable that a number of these patients are transferred to asylums or are heavily sedated as instances of acute delirium or hysteria. Objective anatomic findings are often missing and the only diagnostic criterium is overlooked because the color of the urine is not given enough importance in modern medical hospital diagnosis. At this stage of the disease the patient is usually brought to a surgical ward and many of these unfortunate patients are subjected to multiple operations as the surgeon does not dare to take the responsibility for not opening the abdomen. The abdominal operations performed are indicated in Figure 3. The presence of slight fever, leukocytosis and tachycardia are all compatible with an attack of porphyria but their importance is naturally difficult to interpret in a given case. On the whole it may be said that the pulse rate is by far the best indication of the state of intoxication. Not until the pulse rate has come down is the patient to be regarded as safely on the way to remission. Vomiting may be an alarming symptom as it leads to electrolyte derangements that may become difficult to compensate. The tendency towards oliguria may be accentuated by the vomiting and large amounts of parenteral fluid should then be given. The volume of the urine should always be kept high. The different types of symptoms seen in our material of 233 well analyzed Swedish cases are indicated in Table III.

I shall not enter upon a lengthy description of all the possible symptoms but concentrate upon one of the most important groups from the prognostic point of view, namely the nervous disturbances. It has already been stressed that severe mental derangement is a not unusual finding (28 per cent), usually in association with colic. In a few patients this mental confusion dominates the picture and there are also rare instances in which a comatose state develops. The most important type of neurologic disturbance, however, is a paralysis of skeletal muscles. This paralysis was usually described as being of the so-called acute ascending (Landry) type. The present author has shown [*4*] that this is an interesting example of a scientific cliché that is passed on from one author to another. Of one hundred patients with pareses not one showed a typical ascension of the paralysis. The pareses are scattered quite irregularly and no definite pattern can be made out. They may develop as slight feebleness, often accompanied by aching and tenderness of the muscles. Sometimes this stage is transitory and restitution may occur quickly. The symptom is quite common even when no paresis develops. In severe cases, however, widespread paralyses occur and I have seen patients with complete paralysis of the limbs, as well as involvement of several cranial muscles. Respiration is then very shallow but usually suffices to keep the patient alive. Before



(A) Hysteria (B) Normal (C) AIP

FIG. 3. Number and type of operations in forty-three female patients with AIP from Sweden. (C) o means probably correct diagnosis. "Appendicitis," gynecologic diagnoses and "ileus, volvulus, ulcus" predominate. "Normal" and "hysteria" quoted from COHEN, *J. A. M. A.*, 1953.

restitution sets in these patients become maximally emaciated and look like skeletons. In spite of this fact restitution may become practically complete. Death usually occurs from respiratory paralysis but I have also seen patients die in coma or from incurable cachexia. A colleague who has seen many cases of porphyria in Lapland made the rather striking remark: "When somebody dies from hysteria in this province she usually suffers from acute porphyria."

It is remarkable that few symptoms implicating the sensory nervous system have been observed. Pain (headache, neuralgia) is not uncommon. Most interesting in this respect is the occurrence of sudden amblyopia which may be transitory. Cutaneous senses are usually not impaired. Epileptic fits occur in about 10 per cent of the cases. If interested in the neurologic and psychiatric aspects of the disease one should study reviews of this subject [*52,53*].

Treatment. This paper will not go into details regarding the treatment of porphyria. Prognosis has been much improved by recently devised therapeutic regimens. Nineteen members of our largest porphyria family in Sweden died from porphyria during the period from 1927 to 1937 whereas only two died in the next ten year period, indicating that therapeutic and, above all, prophylactic measures may be important. The principles are the following: Oliguria is often present, sometimes together with azotemia. Fluids should be forced parenterally if vomiting is severe in order to maintain a diuresis of 2 to 3 liters. Severe constipation should be counteracted with enemas, saline, cathartics and prostigmine. Pains and restlessness can be treated with opiates. Under no circumstances should barbiturates or other synthetic sedatives be used. Recently Watson [*54*] has advocated the use of chlorpromazine and similar substances. We have tried them in several cases with good results and

TABLE III*
SYMPTOMS OF ACUTE PORPHYRIA

Symptoms	Number of Cases	Per cent
Colics	198	85
Vomiting	140	59
Constipation	112	48
Localized pareses	99	42
Hypertension	93	40
Fever	86	37
Tachycardia	66	28
Delirium	65	28
Hysteria	38	16
Areflexia	37	16
Cephalalgia	32	14
Apathy	26	11
Convulsions	24	10
Neuralgia	24	10
Azotemia	24	10
Sensory disturbances	22	9
Proteinuria	22	9
Diarrhea	21	9
Pigmentation	16	7
Oliguria	16	7
Leukocytosis	16	7
Transitory amaurosis	9	4
Jaundice	8	3
Diplopia	7	3

* Unpublished data from B. H. Aronsen.

have seen no harmful effects. In regard to the dangers of synthetic drugs, the reader is referred to papers in the Ciba Symposium [*15*] and the Roche meeting [*60*]. One of the most important facets in the treatment of patients with AIP is to avoid all barbiturates and other sleeping pills.

FIG. 4. Map of Sweden showing the known cases of acute porphyria from each province (upper figure), incidence of different genes at the present moment (first of lower figures) compared to thousands of inhabitants in the same district (last figure). For Stockholm this means seventy-nine known cases from ten different non-related families in 1.5 million inhabitants.

Opiates may be used however. Toxic sulphonal porphyria may be seen in a previous latent porphyria.

Acute intermittent porphyria, AIP, has always been regarded as a rare disease. It is quite obvious that the condition is much more common in certain countries or districts than in others. The map of Sweden that is reproduced in Figure 4 shows the number of affected persons who are descendants of patients living in a certain province. It is easy to see that there are more cases in the northern part of Sweden than in the rest of the whole country, in spite of the fact that the total population in this district is only one-sixtieth of that of the whole country. This is chiefly explained by the fact that one large family with no less than 137 known porphyric subjects lives in the Northern province of Lapland. All the descendants of this family have been considered to originate from the Northern province even if they themselves were born in other parts of the country. In spite of elaborate genealogic work there are a number of families in Northern Sweden that cannot be traced back to this largest family. On this account we therefore also find the largest number of different families with porphyria per 100,000 inhabitants in this same area. This may depend upon the fact that the chances for new mutations are especially great in this part of the country or also that our genealogic work-up has not been complete enough. This question will be discussed later. It is an important fact, however, that whereas the incidence of AIP patients in this part of the country is about 1:1000, their incidence in the rest of Sweden is 1.5/100,000. This high frequency is in itself quite probably due to the interest that the Swedish medical profession has shown in this disease during the last twenty years. In recent years Dean and Barnes [*50*] have studied several large families with AIP in South Africa. It is evident that the penetrance of the dominant gene in these families was of the same kind as in our great family from Northern Sweden. Under such circumstances the presence of *one* porphyria family with many descendants may alter the frequency of porphyria in a whole country. Exogenous factors are probably of importance for the unusual clinical picture that is described by Dean in some of his cases.

MIXED PORPHYRIA

The most difficult group of cases comprises what Brunsting [*32*], Watson [*33*] and others have called mixed porphyria. These patients may start with complaints indicating acute intermittent porphyria and cutaneous porphyria develops later. Some observations are difficult to interpret as many patients with PCT have signs of liver cirrhosis, and "colic" is a common symptom in this disease. More specific for AIP are neurologic symptoms such as acute delirium and generalized paresis. At least three or four

patients in the literature have unquestionably had neurologic signs of porphyria in association with cutaneous manifestations. It is remarkable that such episodes were always accompanied by the excretion of PBG that was otherwise not found in the urine. Most puzzling are the observations by Dean, Barnes, Scott and Grotepass [*55*] and others in South Africa. These authors have shown that typical PCT is comparatively common in some Bantu populations. It is a well known fact that nutritional cirrhosis is also common in South Africa. Alcoholism on the other hand does not seem to play an important part. The combination of liver damage and excessive radiation from the sun may perhaps explain some of these cases [*50*]. It has been shown that intense radiation may increase the output of porphyrins.

Among white persons living in the same African districts a curious cutaneous malady has been described, also occurring in families as a dominant trait. The histories are typical of AIP and death may occur with neurologic symptoms. Data regarding the excretion of PBG have not yet been reported. The involvement of the skin does not seem to be identical with that seen in PCT and it is difficult to determine whether or not the previously mentioned cutaneous changes in the Bantus are similar to those seen in the white persons with AIP. The simplest explanation of this combination would be that in people with acute porphyria, after their liver has been damaged in a certain way that is not too uncommon in South Africa, cutaneous symptoms may develop superimposed upon their AIP. Dean has also stressed the important part played by the strong sunshine. A conclusive discussion of this problem must be postponed until the cases have been published in detail, with photographs and biochemical data.

In Watson's large experience there are also a few patients who have suffered from cutaneous symptoms and in whom AIP has then developed, but the reverse sequence also has been seen [*56,57*]. My own experience is of course limited but I know the case histories of no less than 324 Swedish patients with AIP and have had personal contact with most of the families. In this group I have found no patient with symptoms indicating PCT. This latter disease is by no means unknown in Sweden; I have seen a number of patients, none of whom have had relatives with AIP. I am therefore of the opinion that in our country—as in most others—AIP remains true to type and is entirely separate from the cutaneous porphyrias.

HEREDITY OF THE PORPHYRIAS

It is probable that congenital porphyria is inherited as a recessive trait; this seems to have been definitely proved in cattle. In regard to the other cutaneous porphyrias, protocoproporphyria may obviously be dominant [*38*]. Whether or not other modes of inheritance also occur cannot be definitely settled before we have more observations. The family history reported by Goldberg and Berger [*49*], which is unique thus far, indicates recessive heredity in this special disturbance even if the heterozygotes were biochemically recognizable.

Acute intermittent porphyria has been known to occur in families since the beginning of the 1930's [*4,58*]. Concordant appearance in uniovular twins is usually regarded as a sign of heredity. We have one such couple in the Swedish case material. Both sisters fell ill at about the same time, when they were thirty-five years of age. One died, the other recovered and has since been free from acute symptoms even though her urine still shows a marked increase in PBG but curiously enough not in ALA.

It would, of course, be of great interest if we could find an expression for the number of "independent genes," that is, the number of new mutations in the same locus causing porphyria in different parts of Sweden. Such an investigation must be based on the assumption that porphyria is always a genetically determined disease with at least two members in one family showing the biochemical signs characteristic of AIP. This is impossible however. It must be remembered that negative biochemical findings in a family do not exclude a genetic basis for a disorder. There are three reasons for this. (1) The propositus himself may be the first mutation. (2) Even when there are several siblings chance might explain why only one is a carrier. (3) We know that even persons who have suffered from AIP for long periods and have had acute episodes may stop excreting PBG, and thus become "normal" even after thorough biochemical investigation. Such intermittent excretors of PBG were described in 1937 [*4*]. It is probable that such cases will become much rarer when they can be examined with the aid of modern refined technics for the demonstration of PBG and ALA.

Let us now assume that AIP always occurs as an inborn error of metabolism. Let us further examine the pedigrees of all such patients in Sweden. This has been accomplished for the total number of 321 Swedish cases. One hundred and thirty-seven were found to belong to the same family. The three next largest families comprise fourteen, twelve and nine porphyric members. They also come from Lapland, three of them are ethnically Laps. This seems interesting when we remember the correlation between Negroes and pathologic hemoglobins or Levantines and thalassemia. It could perhaps be imagined that the "penetrance" of the porphyria gene is strongest in persons with Lap ancestry. The presence of modifying genes may be assumed. In spite of very thorough search nearly all porphyria families in the rest of the country are smaller: one seven–, two six–, two five–, five four–, three six–, six two–member porphyria families are scattered over the country. *This would mean that 242 of the 321 porphyria cases in Sweden definitely are familial.* It has already been pointed out that familial incidence of a porphyrin disturbance does not necessarily mean that the disorder is genetically determined. I do not know of any case, however, in which each of two unrelated parents suffered from AIP. The only example from the literature that could possibly be adduced as indicating common exogenous influences (where endogenous factors could be excluded) is the old observation by Ranking and Pardington in 1890 that two unrelated persons living in the same house both fell ill with porphyria. In my material from Sweden porphyria has often been noted to occur in successive generations, the record being four generations. It has also occurred in first cousins, uncles and aunts and second cousins of propositi. It therefore seems definitely proved that the majority of cases in Sweden have a genetic basis for their disease.

Assuming that every patient with acute intermittent porphyria in Sweden has a genetic basis for his disease, we have worked out pedigrees for every known case. At present, 4,450 relatives of porphyric subjects have been identified and indexed. In about twenty-five persons it was impossible to obtain a reliable pedigree (some important ancestor could not be traced correctly, family data regarding the patient's birth place were faulty, etc.). The ancestry of 294 cases has been investigated back more than one hundred years. In several families the pedigree could be traced back for approximately 300 years. When it was found that seemingly unrelated cases had a common ancestry we only investigated the section of the genealogy in which the porphyria gene was present. In this way we have worked out a number of pedigrees in which it could be assumed with certainty that there were carriers of the gene who had not shown signs of porphyria. We have defined as a carrier a person whose children, nephews or siblings have been definitely proved to be porphyric. Such persons are found in large numbers and their biochemical status may be examined, their state of health may be ascertained in retrospect, when they are dead, and their age at death may be learned from the churchbooks. In most instances the symptoms at death have also been noted down by the parish clergyman. An analysis of these data would require too much space here but it may be said that no facts have been found that might indicate the presence of unrecognized porphyria in these earlier generations. A discussion of this interesting fact has been given in another paper [*59*].

The present author has collected the total Swedish clinical material and in our data nothing contradicts the assumption that porphyria is inherited as a dominant trait. In a number of sibships I have been able to examine the urine of many siblings for the presence of PBG and uroporphyrin, and only one member has been found to be affected. It is not possible, however, to make any deductions from such facts as intermittent excretion of PBG and consequently of uroporphyrin, as is well known. It has been established that in patients who have been biochemically diagnosed as having latent porphyria manifest porphyria developed later. Most manifest porphyric patients again become latent after the attack and can be recognized only biochemically. I have seen cases, however, in which even excretion of PBG must have stopped, at least temporarily. Such irregularities make it impossible at the present moment to diagnose all carriers of the trait and separate them from the non-carriers. The ratio between these two groups in a sibship can therefore not yet be determined. (Table IV.)

An abnormal frequency of consanguineous marriages in the parental couples of AIP patients might have been a point in favor of a recessive heredity; such consanguinity was not found to be abnormally common however.

TABLE IV*

EXCRETION OF PBG AND ALA IN MG./24 HR. (NORMAL <1 AND <3)

Subject	Data	PBG	ALA
Acute Porphyric Subjects and Relatives			
E. G. ♀	During attacks of colic	51; 73	26; 39
	Later	34	21
Father		0.9	2.7
Mother		0.8	1.8
E. S.-J. ♀	During attacks of colic	80; 87; 39	48; 41; 25
		73; 144	33; 62
Mother (carrier)		9	6
Sister I	No symptoms	1.2	1.9
Sister II	No symptoms	8	5.5
G. H. (surviving uniovular twin)	No symptoms	14; 21; 25; 35	14; 14; 14; 15
Mother	No symptoms	1.2	1.0
Sister I	No symptoms	1.5	0.3
Sister II	No symptoms	1.0	9.0
Brother	No symptoms	9.0	10
I. S. (Fig. 7.)	No attacks of colic	3.6	20
Child, son		0.4	1
Child, daughter		0.5	1
I. A. (Case 1)		24	68
N. L. (Brother Case 70)		30	34
R. S.	No attacks of colic	28	12
Boy I		0.6	3
Boy II		0.7	2
R. O.		0.3	1
Three other typical patients with acute porphyria in remission showed increased values			
Mixed Case			
D.		3.3	11
Protocoproporphyria			
Girl (light sensitivity). feces: protoporphyria coproporphyria increased		Normal	Normal
Father		Normal	Normal
Mother		Normal	Normal
Typical PCT (non-alcoholic)			
D. H., four different specimens	Uroporphyrin positive	Normal	Normal

* Unpublished data from B. H. Aronsen.

The occurrence of several generations with afflicted members is, of course, strong evidence for a dominant heredity. This was noted in a great many families but it is obvious that the penetrance of a gene varies considerably, as there are many undoubted carriers of the gene

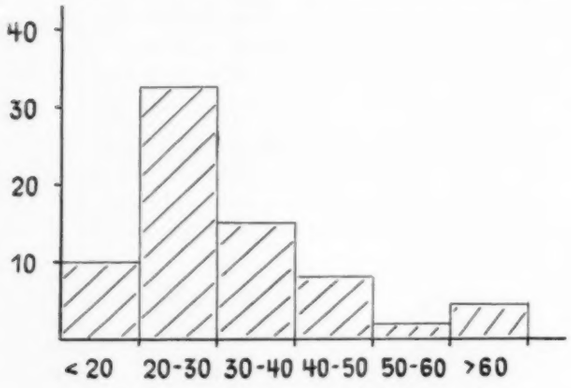


FIG. 5. Age at death from AIP (Sweden).

who have never had any symptoms even at a mature age. The life expectancy of a patient with manifest porphyria was not very good in the beginning of this century, as is clearly seen in Figure 5. It does not seem improbable that the mortality from the disease was less during the nineteenth century in Sweden.

Data from Sweden thus definitely prove that acute porphyria must often be inherited as a dominant trait. No facts can be collected to prove the occurrence of acquired acute porphyria and most families with many siblings that could be investigated showed that the propositus was not the only member exhibiting porphyria. It therefore seems probable that acute porphyria is an inborn error of metabolism of the same kind as the other disorders in this interesting group of diseases. Representative pedigrees are shown in Figures 7 through 12.

What really is inherited in acute porphyria? From our work on latent porphyric subjects [*4*] and on the conversion of porphobilinogen to uroporphyrin [*9,60*], it becomes clear that porphobilinogen is primarily excreted both by the carrier and the manifest porphyric subjects. Uroporphyrin is formed secondarily and this condensation is enhanced by an acid reaction and an elevated temperature (boiling). (See color plates in [*60*].) The presence of the colorless chromogen in the body obviously accounts for the absence of light sensitivity. Experimental work seems to have shown that the porphyrins might well account for the light

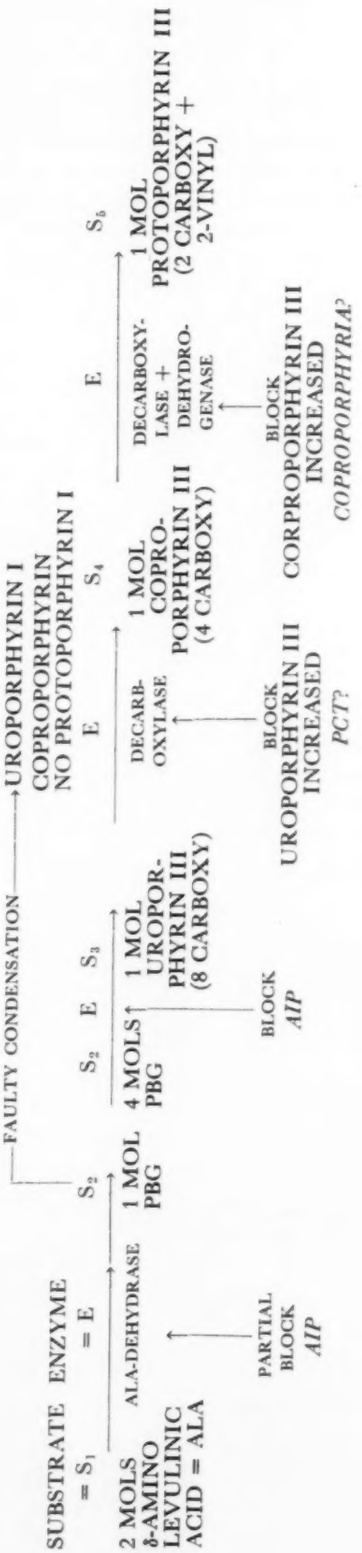


FIG. 6. Schema of enzyme blocks resulting in congenital porphyria.

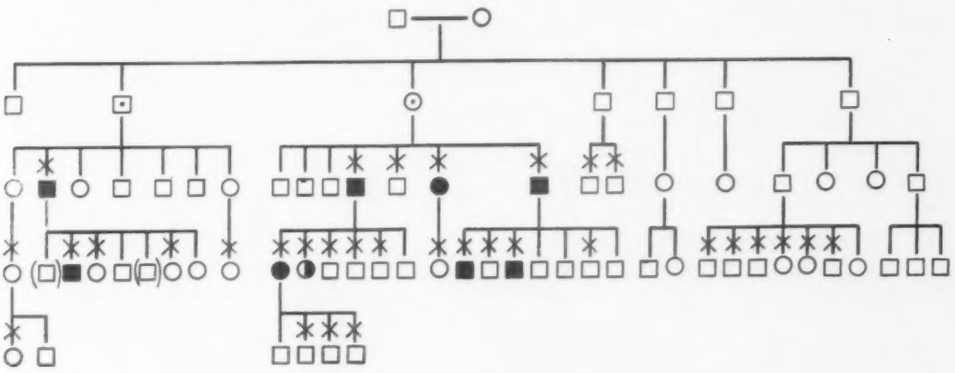


FIG. 7.

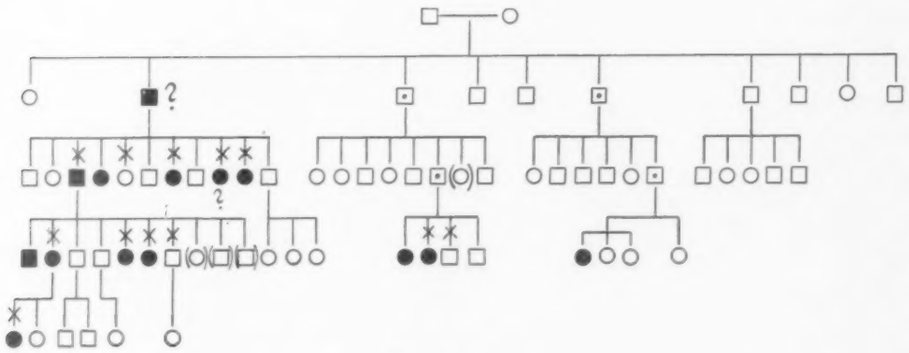


FIG. 8.

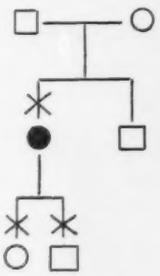


FIG. 9

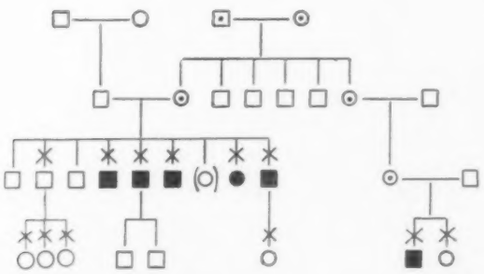


FIG. 10.

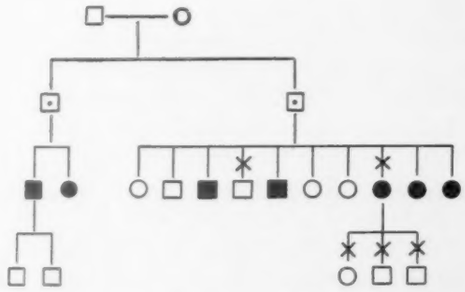


FIG. 11.

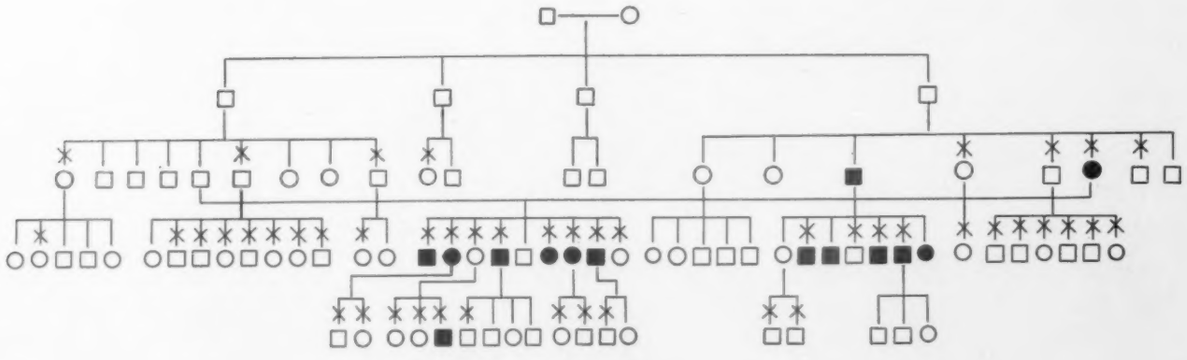


FIG. 12

FIGS. 7–12. Pedigrees of AIP families from Sweden. □ indicates genetically certain carrier of trait but without symptoms. × indicates those examined chemically.

sensitivity in the cutaneous porphyrias but not for the varying symptoms in acute porphyria. It is an intriguing question, however, why the symptoms of lead poisoning should resemble those of acute porphyria: Porphyrinuria, colic and sometimes paresis or even encephalopathy; PBG has, however, never been identified in the urine in lead poisoning. It is also remarkable that the patients with protocoproporphyria in whom colic, psychosis and paralysis developed showed only porphobilinogen in the urine during this stage. It seems tempting to relate some of the symptoms to the porphobilinogen even if animal experiments so far have been negative [*61,62*]. The present author made a rather wild conjecture regarding a possible mechanism when he pointed out that coniin is a β-propylpiperidin and thus may be similar to PBG. We know from as long ago as the time of Plato that this classic poison causes motor paralysis. Whether or not a compound with a similar configuration could have similar effects is difficult to determine at present.

TENTATIVE CLASSIFICATION

Congenital Porphyria. Present from birth; marked light sensitivity. Mainly uroporphyrin I and coproporphyrin. No PBG. Never symptoms of AIP.

PCT Symptomatica. Alcoholic cirrhosis: Begins after forty years of age; moderate light sensitivity. No PBG. Never AIP. *Bantu cirrhosis:* Same symptoms as in alcoholic cirrhosis. *Exceptional cases:* (Tiong Hoo [*38*], hepatoma; Hamminga [*40*], liver tumor; own case.) Symptoms same as those for alcoholic cirrhosis.

PCT Hereditaria. Protocoproporphyria: Begins at about puberty. Reciprocity theory of Rimington: always protoporphyrin and coproporphyrin in feces. Light sensitivity only when porphyrin appears in the urine. Uroporphyrin and PBG in liver attacks, only then colic, pareses.

Acute Intermittent Porphyria. Probably always dominant in heredity. PBG and ALA in urine. Latent or manifested by severe attacks. Mainly in South Africa: in some acute porphyric subjects nutritional or toxic factors may give cutaneous symptoms.

Acknowledgment: The genealogic part of this work was aided by grants from the Rockefeller Foundation in New York. My thanks to many colleagues who aided in this work by furnishing invaluable clinical data. Above all I wish to thank Director General, Dr. Arthur Engel, former head of the General Hospital in Boden, Dr. Gösta Neander of Uppsala, Nurse Ragnhild Blomquist, Dr. Birgitta Haeger-Aronsen of Malmö and Mrs. Anita Stentorp of Malmö.

REFERENCES

1. Watson, C. J. and Larson, E. A. The urinary coproporphyrins in health and disease. *Physiol. Rev.*, 27: 478, 1947.
2. Dobriner, K. and Rhoads, C. P. The porphyrins in health and disease. *Physiol. Rev.*, 20: 416, 1940.
3. Günther, H. Hämatoporphyrie, in Schittenhelm, A. Handbuch der Krankheiten des Blutes und der blutbildenden Organe, vol. 2, Berlin, 1925. Julius Springer.
4. Waldenström, J. Studien über Porphyrie. *Acta med. Scandinav.*, Suppl. 82, 1937.
5. Schmid, R., Schwartz, S. and Sundberg, D. Erythropoietic (congenital) porphyria: a rare abnormality of the normoblasts. *Blood*, 10: 416, 1955.
6. Fischer, H. Über das Kotporphyrin. *Ztschr. physiol. Chem.*, 96: 148, 1915.
7. Fischer, H. Über das Urinporphyrin. *Ztschr. physiol. Chem.*, 95: 34, 1915.
8. Watson, C. J. and Schwartz, S. A simple test for urinary porphobilinogen. *Proc. Soc. Exper. Biol. & Med.*, 67: 393, 1941.
9. Waldenström, J. and Vahlquist, B. Studien über die Entstehung der roten Harnpigmente (Uroporphyrin und Porphobilin) bei der akuten Porphyrie aus ihrer farblosen Vorstufe (Porphobilinogen). *Ztschr. physiol. Chem.*, 260: 189, 1939.
10. Westall, R. G. Isolation of porphobilinogen from the urine of a patient with acute porphyria. *Nature, London*, 170: 614, 1952.
11. Cookson, G. H. and Rimington, C. Porphobilinogen. *Biochem. J.*, 57: 476, 1954.
12. Neuberger, A., Muir, H. M. and Gray, C. H. Biosynthesis of porphyrins and congenital porphyria. *Nature, London*, 165: 948, 1950.
13. Shemin, D. and Wittenberg, J. The mechanism of porphyrin formation: The role of the tricarboxylic acid cycle. *J. Biol. Chem.*, 192: 315, 1951.
14. Shemin, D. and Russell, C. S. δ-Amino levulinic acid: its role in the biosynthesis of porphyrins. *J. Am. Chem. Soc.*, 75: 4873, 1953.
15. Shemin, D. The Succinate-Glycine Cycle; the Role of δ-Aminolevulinic Acid in Porphyrin Synthesis, Porphyrin Biosynthesis and Metabolism, p. 4. Ciba Foundation. London 1955. J. & A. Churchill, Ltd.
16. Granick, S. Enzymatic conversion of δ-amino levulinic acid to porphobilinogen. *Science*, 120: 1105, 1954.
17. Bogorad, L. and Granick, S. The enzymatic synthesis of porphyrins from porphobilinogen. *Proc. Nat. Acad. Sc.*, 39: 1176, 1953.
18. Granick, S. and van den Schrieck, H. G. Porphobilinogen and δ-aminolevulinic acid in acute porphyria. *Proc. Soc. Exper. Biol. & Med.*, 88: 270, 1955.
19. Schmid, R., Schwartz, S. and Watson, C. J. Porphyrin content of bone marrow and liver in the various forms of porphyria. *Arch. Int. Med.*, 93: 167, 1954.

20. Schmid, R., Schwartz, S. and Watson, C. J. Neuere Ergebnisse auf dem Gebiete der Porphyrien. *Acta haemat.*, 10: 150, 1953.
21. Garrod, A. E. Inborn Errors of Metabolism, 2nd ed. London, 1923. H. Frowde.
22. Findlay, G. H. and Barnes, H. D. Congenital porphyria, hydroa aestivale and hypertrichosis in a South African Bantu. *Lancet*, 2: 846, 1950.
23. McCall Anderson, T. Hydroa aestivale in two brothers complicated with the presence of haematoporphyrin in the urine. *Brit. J. Dermat.*, 10: 1, 1898.
24. Sato, A. and Takahashi, H. A new form of congenital hematoporphyria: oligochromemia porphyrinuria (megalosplenica congenita). *Am. J. Dis. Child.*, 32: 325, 1926.
25. Kitagawa, K. Über Hämatoporphyria congenita "Hans Günther" und ihre experimentelle Untersuchung. *Jap. J. Dermat. Urol.*, 27: 43, 1927.
26. Matsuoka, K. Über Haematoporphyria congenita. *Jap. J. Dermat. Urol.*, 28: 38, 1926.
27. Hernando, T. La porphyrie: ces manifestations digestives, cutanées et oculaires. *Biol. méd., Paris*, 36: 293, 1938.
28. May, E., Bloch-Michel, M., Poncet-Guaret and Tournier, P. Porphyrie familiale (maladie de Gunther) dans la même fratrie. *Bull. Soc. méd. hôp. Paris*, 64: 340, 1948.
29. Jørgensen, S. K. and With, T. K. Congenital porphyria in swine and cattle in Denmark. *Nature, London*, 176: 156, 1955.
30. Rimington, C. Haems and porphyrins in health and disease. *Acta med. Scandinav.*, 143: 161, 1952.
31. Szodoray, L. and Sümagi, S. Über die Nosologie der chronischen kutanen Porphyrie. *Dermatologica*, 90: 224, 1944.
32. Brunsting, L. A. Observations on porphyria cutanea tarda. *Arch. Dermat. & Syph.*, 70: 551, 1954.
33. Watson, C. J. Porphyria. Advances in Internal Medicine, vol. 6. Chicago, 1954. Year Book Publishers.
34. Borda, J. M. Cited by Brunsting.
35. Bolgert, M., Canivet, J. and Le Sourd, M. La porphyrie cutanée de l'adulte; étude de neuf cas et description. *Semaine d. hôp. Paris*, 29: 1587, 1953.
36. Tappeiner, S. and Tirschek, H. Das Syndrom der aktinisch-traumatischen bullösen Porphyrindermatose. *Arch. f. Dermat. & Syph.*, 196: 65, 1953.
37. Paul, K. G., Engström, A. and Engfeldt, B. Localization of porphyrins in bone tissue in a case of porphyria cutanea tarda. *J. Clin. Path.*, 6: 135, 1953.
38. Tiong, Hoo, T. Beschouwingen over de porphyria cutanea tarda, Dissertation, Haag, 1956. Excelsior. (Papers in English by this author are in press).
39. Waldenström, J. In: Avery Jones' Modern Trends in Gastroenterology II. (In press.)
40. Hamminga, H. Porphyria with light sensitivity. *Nederl. tijdschr. v. geneesk.*, 95: 696, 1951.
41. Wells, G. C. and Rimington, C. Studies on a case of porphyria cutanea tarda. *Brit. J. Dermat.*, 65: 337, 1953.
42. Macgregor, A. G., Nicholas, R. E. H. and Rimington, C. Porphyria cutanea tarda. *Arch. Int. Med.*, 90: 483, 1952.
43. Discombe, G. and Treip, C. S. Cutaneous manifestations of porphyria. *Brit. M. J.*, 2: 1134, 1953.
44. Gray, H., Rimington, C. and Thomson, S. Case of chronic porphyria associated with recurrent jaundice. *Quart. J. Med.*, 17: 123, 1948.
45. Calvert, R. J. and Rimington, C. Porphyria cutanea tarda in relapse: a case report. *Brit. M. J.*, 11: 1131, 1953.
46. Hijmans Van Den Bergh, A. A. and Grotepass, W. Ein bemerkenswerter Fall von Porphyrie. *Wien. klin. Wchnschr.*, 21: 830, 1937.
47. Taylor, I., Solomon, M. L., Weiland, G. S. and Figge, F. H. J. Chronic porphyria. *J. A. M. A.*, 131: 26, 1946.
48. Barnes, H. D. Porphyria in the Bantu races on the Witwatersrand. *South African M. J.*, 29: 781, 1955.
49. Berger, H. and Goldberg, A. Hereditary coproporphyria. *Brit. M. J.*, 2: 85, 1955.
50. Dean, G. and Barnes, H. D. The inheritance of porphyria. *Brit. M. J.*, 2: 89, 1955.
51. Kuske, H. Zum Problem der Hautveränderungen bei Porphyrie. *Dermatologica*, 92: 149, 1946.
52. Garcin, R. and Lapresle, J. Manifestations nerveuses de la porphyrie. *Semaine d. hôp. Paris*, 26: 3404, 1950.
53. Waldenström, J. Neurological symptoms caused by so-called acute porphyria. *Acta psychiat. et neurol.*, 14: 375, 1939.
54. Watson, C. J. Personal communication.
55. Scott, F. P. and Grotepass, W. Über verschiedene Formen von Porphyrie bei Weissen und Bantus in Südafrika. *Med. Klin. Berlin*, 51: 679, 1956.
56. Nesbitt, S. and Watkins, C. H. Acute porphyria. *Am. J. M. Sc.*, 203: 74, 1942.
57. Turner, W. J. and Obermayer, M. E. Studies on porphyria. *Arch. Dermat. & Syph.*, 37: 549, 1938.
58. Waldenström, J. Studies on the incidence and heredity of acute porphyria in Sweden. *Acta genet.*, 6: 122, 1956.
59. Roche-Symposium in Malmö, 1956.
60. Waldenström, J. and Vahlquist, B. Studies on the excretion of porphobilinogen in patients with so-called acute porphyria. *Acta med. Scandinav.*, 117: 1, 1944.
61. Waldenström, J. and Wendt, S. Tierexperimentelle Studien über den Porphyrinstoffwechsel. *Ztschr. physiol. Chem.*, 259: 157, 1939.
62. Goldberg, A., Paton, W. D. M. and Thompson, J. W. Pharmacology of the porphyrins and PBG. *Brit. J. Pharm. & Chemotherap.*, 9: 91, 1954.
63. Mauzerall, D. and Granick, S. The occurrence and determination of δ-aminolevulinic acid and porphobilinogen in urine. *J. Biol. Chem.*, 219: 435, 1956.

Cystinuria

H. Harris, M.D., *and* E. B. Robson, M.D.
London, England

In 1810 Wollaston described a "new species of urinary calculus." During the following 100 years many further examples of the same kind of stone were identified and it was shown that they were composed almost entirely of the amino acid, cystine. Patients in whom such stones formed were found to be excreting large amounts of cystine continuously in their urine. The disorder was frequently familial and was recognized to be in some way genetically determined.

It is now known that cystine is not the only amino acid excreted in unusual quantities by patients in whom cystine calculi form. The basic amino acids, lysine, arginine and ornithine, are also regularly present in the urine in grossly abnormal amounts (Ackermann and Kutscher [*1*], Yeh et al. [*26*], Dent and Rose [*9*], Stein [*24*], and Harris, et al. [*18*]). The quantities involved are remarkable. Stein [*24*], for example, in five cases found that an average of 0.73 gm. of cystine, 1.8 gm. of lysine, 0.83 gm. of arginine and 0.37 gm. of ornithine were excreted per day. This massive amino aciduria is continuous and probably persists with little change throughout life. Furthermore, the abnormality appears to be highly specific for these four substances.

Earlier reports that the diamines, cadaverine and putrescine, sometimes occurred in large amounts in cystinuric urines have not been confirmed when modern analytic methods were applied to fresh urine samples. It is possible that they represented artefacts due to infection or that the substances were misidentified.

Cystine may also be excreted in unusual amounts in a number of other genetically determined conditions. Among these may be mentioned cystinosis, the different variants of the De Toni-Debré-Fanconi syndrome, and Wilson's disease or hepatolenticular degeneration.

In the past, cystinosis has been confused with the type of cystinuria just described. It is now clear, however, that these disorders may be sharply differentiated on clinical, biochemical and genetic grounds [*10*]. In particular, the amino aciduria found in such conditions is of a much more generalized type, involving some ten or more amino acids, and cystine stone formation rarely if ever occurs. It is convenient, therefore, to reserve the term "cystinuria" for the clinical condition involving recurrent cystine calculus formation, as described by Wollaston, and now known to be characterized by the abnormal excretion of cystine, lysine, arginine and ornithine.

The term "cystine-lysinuria" has also occasionally been used in recent years to designate the condition. This is unsatisfactory because, as will be seen later, true cystine-lysinuria, when it occurs without concomitant arginine and ornithinuria, is only rarely associated with stone formation. The incorporation of all four amino acids in the name for the disease is somewhat cumbersome, and now that the situation has been clarified it seems simplest to retain the classic term cystinuria.

The loss of the four amino acids, even in these considerable amounts, does not lead to any obvious nutritional disturbances in the patients, providing that they are on a normal diet with an adequate protein content. All the clinical features of the condition can be attributed simply to the complication of recurrent formation of stones in the renal tract. Apart from the tendency to calculus formation and consequent obstruction in the renal tract, these patients may remain remarkably well. Surprisingly enough, chronic renal infection does not usually occur. The prime cause of the stone formation is undoubtedly the high urinary concentration of cystine. Cystine is one of the least soluble of amino acids and in urine between pH 5 and 7 it can be kept in solution only to the extent of 300 to 400 mg./L. [*13*]. In patients excreting between 0.5 gm. and 1 gm. of cystine per day, the concentration of this substance may frequently reach saturation level, particularly at night when the urine passed is most concentrated. Consequently, the cystine will tend to come out of solution and this will lead to calculus formation. Lysine, arginine and ornithine, on the

other hand, are freely soluble and therefore do not become incorporated into the stones.

Diagnosis rests on the demonstration of grossly abnormal amounts of the four amino acids in the urine. Useful routine qualitative tests are the cyanide/nitroprusside test for cystine, and two dimensional paper chromatography for amino acids [*9*]. The three basic amino acids may also be demonstrated easily by ionophoresis in filter paper at pH 11.5 [*17,18*]. The abnormality is so gross that qualitative results by these methods are adequate for most purposes. Quantitative analyses are rather more difficult to carry out and are not necessary in ordinary clinical practice.

THE NATURE OF THE LESION

Until recently it had been assumed that cystinuria represented a disorder in the intermediary metabolism either of cystine itself or of the other sulphur-containing amino acids, cysteine and methionine [*4,15*]. The exact nature of the postulated "block" in the metabolism of these substances had, however, always remained obscure. The demonstration that lysine, arginine and ornithine were equally involved introduced further complications, because of the absence of any obvious specific inter-relation between the intermediary metabolism of these amino acids and the sulphur-containing ones.

An alternative hypothesis was put forward by Dent in 1949 [*8*], when he suggested that the abnormality in cystinuria was essentially renal in character. If the renal tubule cells were unable to reabsorb the cystine, lysine, arginine and ornithine normally present in the glomerular filtrate, these substances would appear in large amounts in the urine and the various features of the disease could be readily explained. A considerable body of evidence has now accumulated in favour of this hypothesis.

If cystinuria arises from some kind of block in the intermediary metabolism of cystine one would expect cystine to accumulate in the body fluids and the plasma level should be considerably higher than in normal people. If, however, cystinuria was a purely renal abnormality no such rise in plasma cystine would be expected. Similar arguments also hold for the other amino acids excreted in abnormal amounts. The plasma cystine level has now been measured in a number of cystinuric patients by a variety of different methods [*11,12,14,23,25*]. It is, in fact, not elevated, as would have been expected on the metabolic block hypothesis but is, if anything, slightly less than normal, as may be anticipated on the renal hypothesis. The same seems to be true for lysine, arginine and ornithine [*2,9,23,25*], although so far only a few plasma determinations of these amino acids have been carried out.

Estimates of the renal clearances of cystine [*12*] and of lysine, arginine and ornithine [*2*] have shown that all are grossly elevated compared with the normal. In the case of cystine, the clearance values are of the same order of magnitude as the expected rate of glomerular filtration. In one subject in whom the inulin and cystine clearances were measured simultaneously, close agreement was obtained. The clearances for lysine, arginine and ornithine are probably somewhat less than the rate of glomerular filtration [*2*]. This suggests that the reabsorption defect, although gross, is not complete.

The renal hypothesis implies that in normal persons the tubular reabsorption of cystine, lysine, arginine and ornithine involves at least one step which is common to and specific for these four amino acids, and that in cystinuria this process is in some way defective. If this is so, one may expect that if the plasma concentration of one of these amino acids were artificially raised to high levels in a normal person, then, as a result of overloading the reabsorptive mechanism, increased excretion of the other three amino acids would occur. This phenomenon has recently been demonstrated by Robson and Rose [*23*]. They infused 5 gm. lysine intravenously into normal subjects and found that increased excretion of cystine, arginine and ornithine as well as of lysine resulted. There was a corresponding increase in the renal clearances. Little change in the excretion of other amino acids occurred. Similar experiments carried out on cystinuric patients caused little or no increase in the already grossly increased excretion of cystine, arginine and ornithine. This, of course, would be expected on the renal hypothesis.

The results of experiments which have been carried out at different times involving the feeding of cystine, cysteine and methionine to cystinuric subjects have rarely been clear cut and have been variously interpreted [*4,11,12*]. They can probably be most comprehensively understood on the renal hypothesis.

If cystine itself is fed to cystinuric patients there is little or no rise in the plasma cystine level and no appreciable increase in the urinary cystine. It appears, to be absorbed and metab-

olised because an appropriate increase in the urinary sulphate can be demonstrated [*3,11,12,21,22*]. Feeding cystine to normal persons gives substantially the same results, so that there appears to be no peculiarity in the cystinuric patient with respect to his ability to deal with dietary cystine.

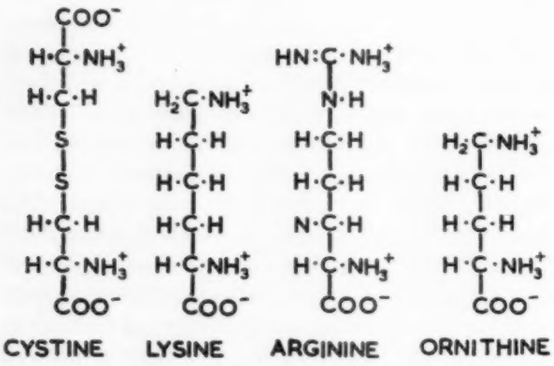


FIG. 1. Formulas of cystine, lysine, arginine and ornithine.

Ingestion of cysteine by cystinuric persons leads to quite different phenomena; their interpretation is, however, made somewhat difficult by the absence of any satisfactory methods for differentiating plasma cystine from cysteine. In general, there is a rapid rise in the plasma level of cystine (or cysteine) and a marked increase of cystine in the urine [*6,7,11,12*]. In all these experiments the urinary cystine output varied with the plasma levels. However, for equivalent plasma levels the cystine excretion was much greater in the cystinuric persons than in the normal subjects. Since the plasma tolerance curves after feeding cysteine to normal and cystinuric subjects were substantially the same, these findings are consistent with the idea that it is the renal tubular reabsorption mechanism that is at fault. Estimates of the renal clearances at different plasma levels following the feeding of cysteine further support this concept. In the cystinuric subjects the clearances did not change significantly with increasing plasma levels but remained virtually constant at a value of the same order of magnitude as the glomerular filtration rate. In normal subjects the clearances were very much lower but did rise significantly with increasing plasma levels [*12*].

The reason cystine ingestion in both normal and cystinuric subjects fails to lead to any marked change in plasma level, whereas cysteine leads to a rapid elevation in plasma level, remains uncertain. Dent and his colleagues [*11,12*], suggest that this is due to different rates of absorption, probably depending on the relative solubilities of the two substances. Cysteine, being readily soluble, is likely to be rapidly absorbed through the intestinal mucosa and so reach the portal blood in high enough concentrations temporarily to saturate the mechanisms for maintaining a constant blood level. A large dose of cystine, however, will at first remain largely undissolved in the intestinal fluids and so be taken up into the portal blood only slowly and consequently be adequately dealt with by the liver. Earlier workers [*7*], however, had postulated entirely different metabolic pathways for cystine and cysteine.

Various results have been reported after feeding methionine. Earlier workers [*7*] did find an increase in cystine excretion after feeding methionine to cystinuric subjects, while Dent et al. [*11,12*] failed to find this. Such minor discrepancies may be a result of different time scales of the experiments.

In general, none of these feeding experiments suggest that cystinuric subjects differ in any material respect from normal subjects in their intermediary metabolism of the sulphur-containing amino acids. The results, on the other hand, are all consistent with the renal hypothesis.

If it is true that in normal subjects there is a pathway in tubular reabsorption common to and specific for cystine, lysine, arginine and ornithine, and that in cystinuric patients this is defective, then one is confronted with the problem as to what property peculiar to these four amino acids leads to their being handled by renal tubules differently from other amino acids. Some evidence for a common reabsorptive mechanism for lysine and arginine has been obtained experimentally in dogs [*5*]. These particular substances resemble each other and also ornithine closely enough in structure and chemical properties to make this association not very surprising. The specific association of cystine with the basic amino acids is, however, rather unexpected. Dent and Rose [*9*] suggested on the basis of the structural formulas (Fig. 1) that the important similarity might be the occurrence of two positively charged amino groups separated by a chain of 4 to 6 atoms. The transport mechanism, for example, might involve the combination of these positively charged amino groups with similarly spaced negatively charged carboxyl groups projecting from alternate glutamate or aspartate residues on a protein surface.

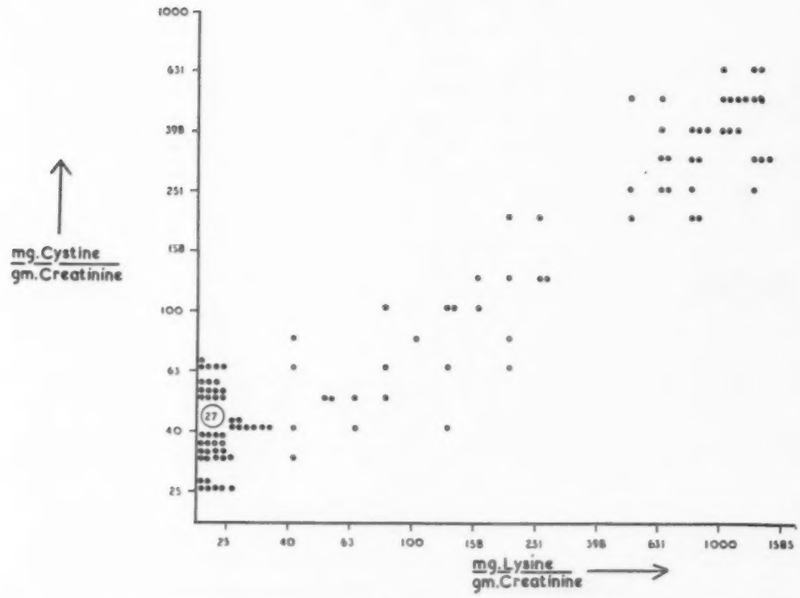


FIG. 2. Cystine and lysine contents of urine from a series of cystinuric patients and their relatives. Each point represents one person. The concentration of cystine and lysine has been related to the creatinine concentration in each sample in order to minimize variations due to different rates of urine formation.

So far there is no direct evidence bearing on this problem, nor has any more plausible explanation been put forward.

FAMILY STUDIES

When urine from apparently healthy relatives of patients with cystine stone formation is examined, it is found that some of them, although clinically in no way abnormal, excrete cystine and lysine in unusual amounts in their urine. Occasionally arginine and ornithine are found as well. Quantitative estimations of cystine, lysine and arginine have been made in a large number of such relatives representing more than thirty families [*16,18,19*]. It is evident that a considerable variation in the amounts of the substances excreted exists among them. Indeed all values may be found, varying between the small amounts encountered in a random sample of the general population and the large amounts encountered in patients with cystine stone formation. Determinations of several samples from particular subjects over periods of two or three years showed that each person had a fairly characteristic level of excretion of these substances.

Among these relatives of cystinuric patients there is a good correlation between the output of cystine and the output of lysine. (Fig. 2.) Differences among persons with regard to their cystine excretion are more or less paralleled by equivalent differences in lysine excretion. On the other hand, abnormal arginine excretion in such persons is found consistently only when the cystine and lysine outputs are relatively high. (Fig. 3.) Below these levels, even though the cystine and lysine outputs may be well above the values found in normal subjects, the arginine output is either within normal limits or is at most only slightly elevated. Apparently, the cystine and lysine excretion must be above a certain threshold before arginine is excreted in excess. The same may be true in the case of ornithine but detailed quantitative results have not as yet been obtained.

Thus in some of these families there are healthy persons with a moderately increased but definitely abnormal excretion of cystine and lysine and with normal arginine and perhaps ornithine outputs. Stones form in such persons only very occasionally, presumably because the urinary cystine concentration rarely rises to saturation levels.

Now, if it is the case that in cystinuric patients with stone formation the essential defect lies in a gross failure of renal tubular reabsorption of cystine, lysine, arginine and ornithine, then the fact that all degrees of cystine and lysine excretion may be found among their apparently

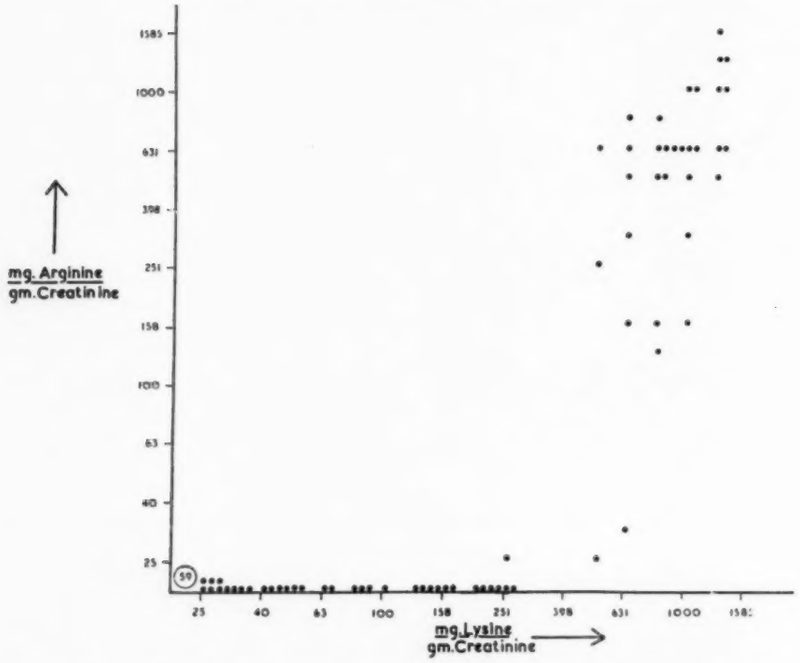


FIG. 3. Arginine and lysine contents of urine from a series of cystinuric patients and their relatives. Each point represents one person. The concentration of arginine and lysine has been related to the creatinine concentration in each sample in order to minimize variations due to different rates of urine formation.

healthy relatives suggests that all degrees of failure of tubular reabsorption may occur. Furthermore, the results on arginine excretion among the relatives indicate that when the reabsorptive capacity for these four amino acids is only partially limited, arginine is reabsorbed preferentially to cystine and lysine. Qualitative results suggest that ornithine may in this respect resemble arginine.

GENETIC ANALYSIS

Genetic analysis has revealed a rather complex situation. If a series of families each containing at least one member with cystine stone formation and massive excretion of cystine, lysine, arginine and ornithine is studied, it can be shown that they are heterogeneous [*16,19*]. In some families persons who are clinically unaffected but have a moderately abnormal excretion of cystine and lysine with little or no detectable abnormality in arginine and ornithine excretion are not found, while in others they are quite frequent. It is possible to divide the families into two groups on this basis.

In the first group of families the intermediate phenotype does not occur, and only two classes of people can be identified; those with a grossly abnormal excretion of cystine, lysine, arginine and ornithine, and those with excretions of these substances within the normal range. The segregation is sharp and without any overlap. The familial distribution here is typical of a Mendelian recessive character. (Fig. 4.) Usually the abnormality occurs in only a single sibship in each family; the parents, children and other relatives are normal, and there is an increased incidence of parental consanguinity. Appropriate calculations indicate that the observed segregation ratios are consistent with those theoretically expected [*19*]. One can conclude that here one is dealing with a rare recessive gene the effects of which are manifest only in homozygous persons. The heterozygotes, that is to say both parents, all the children and a proportion of the other relatives have no detectable abnormality. The persons who carry the abnormal gene in double dose appear to have a severe defect in the tubular reabsorption of the four amino acids concerned, while persons carrying the gene only in single dose can reabsorb these substances as efficiently as other people, at least at normal loads. The type of condition found in this group of families has been called "recessive cystinuria."

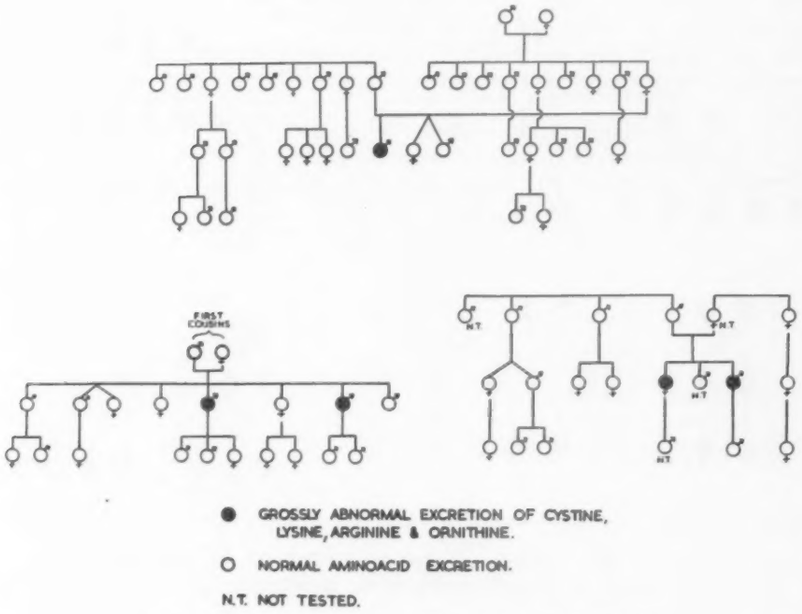


FIG. 4. Typical pedigrees of "recessive cystinuria."

In the second group of families, which is somewhat less common than the first group, the position is more complex. Three types of persons may be identified; those with grossly abnormal excretion of the four amino acids; those with moderate excretion of cystine and lysine but normal or only slightly elevated excretion of arginine and ornithine; and those with normal excretion. The intermediate phenotype is rather variable and some overlap occurs in the three distributions. This is particularly noticeable in the lower ranges of the intermediate class which overlap with the upper range of the distribution of normals, and some difficulty in unequivocal classification occurs. However, there seems little doubt about the reality of these three phenotypes and most persons in such families can be fairly readily assigned to one or the other on the basis of the quantities of the individual amino acids found in the urine. The distribution of these three phenotypes in these families fits well with the idea that the most extreme phenotype in which there is presumably gross failure in tubular reabsorption of all four amino acids, occurs in persons homozygous for the abnormal gene; and the intermediate phenotype with only a moderately defective tubular dysfunction represents the heterozygous persons. (Fig. 5.) Such a hypothesis implies that both parents and all the children of the most severely affected persons are heterozygotes and thus belong to the intermediate phenotype. Similarly, half the uncles and aunts, one of each pair of grandparents and an appropriate proportion of the cousins and other relatives both on the maternal and paternal sides of the family would also belong to the intermediate phenotype. These expectations are in good agreement with the observed findings in these families, providing appropriate allowance is made for some overlapping of the distributions [*19*]. The fact that there is an increased incidence of parental consanguinity among the parents of the affected patients in these families gives further support to the hypothesis. For this and other reasons the type of condition found in this group of families has been called "incompletely recessive cystinuria."

Thus it has become possible to differentiate on genetical grounds two main forms of classic cystinuria; the recessive and the incompletely recessive. In both forms the abnormal homozygotes excrete considerable quantities of the four characteristic amino acids. However, even among such homozygotes considerable variation in the relative proportions of the different amino acids excreted from case to case may be found, and analysis of this variation has revealed further complexities in the genetical situation [*20*].

A convenient index of this variation in pattern of excretion is the ratio of the quantity of lysine to the quantity of arginine in urine samples from

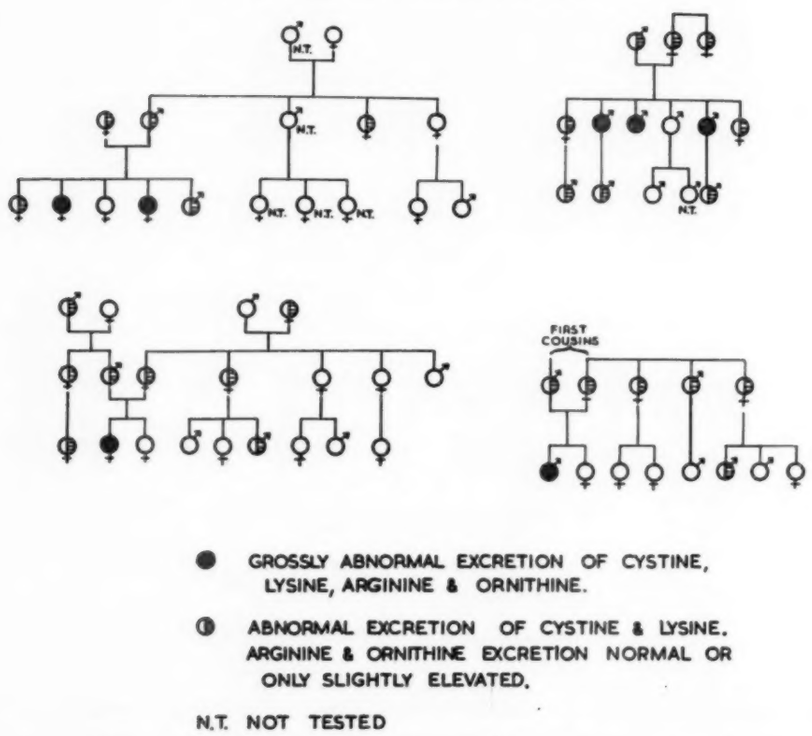

N.T.
FIRST COUSINS
GROSSLY ABNORMAL EXCRETION OF CYSTINE, LYSINE, ARGININE & ORNITHINE.
ABNORMAL EXCRETION OF CYSTINE & LYSINE. ARGININE & ORNITHINE EXCRETION NORMAL OR ONLY SLIGHTLY ELEVATED.
N.T. NOT TESTED

FIG. 5. Typical pedigrees of "incompletely recessive cystinuria."

different homozygous individuals. Values for this ratio as high as 4.2 and as low as 0.8 may occur in different homozygotes and these differences are larger than can be reasonably attributed to experimental error. Furthermore, the variation in the lysine/arginine ratios is much greater among the recessive homozygotes than among the incompletely recessive homozygotes. This difference in variance is highly significant. On the other hand, the mean value for the ratio among the recessive homozygotes is not significantly different from that for the incompletely recessive ones. In the recessive group of homozygotes affected pairs of sibs closely resemble one another in the lysine/arginine excretion ratio, a sib-sib correlation coefficient of the order of 0.90 being found. In the incompletely recessive homozygotes, on the other hand, no sib-sib correlation is demonstrable. All this suggests that the large variation in the pattern of excretion of the recessive group of homozygotes is to a great extent genetical in origin. It seems probable that this group is heterogenous and consists of several distinct conditions caused by separate recessive genes. The affected persons would either be homozygous for one of these genes or heterozygous for two of them. Whether or not the several genes causing "recessive cystinuria" and the genes causing the "incompletely recessive" condition are alleles or at different chromosomal loci remains to be discovered.

CALCULUS FORMATION

Stone formation is a fairly frequent occurrence among persons homozygous for one or another of the genes causing cystinuria. Probably in more than 65 per cent of such persons calculi develop at some time in their lives and in such people stone formation is very often recurrent. In the majority of them the first stone is formed before the age of thirty and in an appreciable fraction of these in infancy and childhood. Thus, although cystine calculi may account for no more than 1 per cent of all renal calculi encountered, they probably form a much larger proportion of all calculi occurring in childhood. The exact proportion however, has not yet been estimated. Stone formation has also occasionally occurred in heterozygotes of the incompletely recessive type who happen to have rather a high level of cystine excretion. This, however, is unusual and the vast majority of heterozygotes remain symptom-free and quite healthy.

Stone formation is, in the main, undoubtedly

TABLE I

DAY AND NIGHT EXCRETION OF CYSTINE IN THE URINE [*13*]

Day	Period	Urine Output (μg./min.)	Cystine Concentration (μg./ml.)	Cystine Excretion (μg./ml.)	pH	Cystine in Solution after Equilibration with Added Cystine (μg./ml.)
First	Day	2.2	275	602	6.2	380
	Night	0.78	690	540	6.1	340
Second	Day	2.2	225	504	6.1	450
	Night	0.49	890	435	6.5	440
Third	Day	1.8	290	530	7.2	500
	Night	0.5	975	486	5.9	310
Fourth	Day	2.4	225	540	...	...
	Night	0.92	575	536	...	...
Fifth	Day	1.7	250	425	...	...
	Night	0.65	610	420	...	...
Sixth	Day	1.8	375	695	...	...
	Night	0.75	745	557	...	...
Seventh	Day	1.9	250	468	...	...
	Night	0.67	700	466	...	...

a function of the cystine concentration attained in the urine. If this should frequently reach saturation level, the patient is at risk. The concentration attained at any time will depend on the rate of excretion of cystine and the volume of urine being formed. Differences in stone formation between homozygotes may therefore depend not only on differences in the absolute rates of cystine excretion but also on individual variations in rates of urine formation. This latter is likely to be of considerable importance in view of the known variations from one person to another in habits of fluid intake. No doubt other factors such as malformations in the structure of the renal tract, infection, transfer to tropical climates with inadequate adjustment of fluid intake to maintain urine volume, and so on, may also play a part in deciding why stones are more prone to form in one homozygote than in another.

What may be of particular significance in stone formation is the diurnal rhythm in urine flow. Most people pass much more concentrated urine at night time than in the daytime; it may well be that most of the stone formation occurs in the night. This possibility is emphasised by the results of an experiment carried out by Dent and Senior [*13*] on a cystinuric patient. Urine collections were made during two periods each day; a daytime period from 6 a.m. to 10 p.m. and a night time period of 10 p.m. to 6 a.m. During this time the patient was allowed to follow her normal habits of food and fluid intake. The volumes of urine passed and their cystine content were measured in each period. (Table I.) The average daytime excretion of cystine was 538 μg. per minute and the night excretion average was 492 μg. per minute. There was thus little difference in the rate of cystine excretion in the two periods. On the other hand, the urine output fell from an average daytime level of 2.1 ml. per minute to an average night time one of 0.67 ml. per minute. In consequence, the concentration of cystine in the night urine was much more than in the corresponding day urine. During part of this experiment solubility studies were also carried out. The addition of solid cystine to the day urines caused an increased concentration in the urine filtrate, while in the night urines the concentration decreased. Thus the daytime urines were under-saturated with cystine and the night time urines were super-saturated.

TREATMENT

A therapeutic approach to the problem of stone formation would seem to depend on either an attempt to decrease the quantity of cystine excreted or increase its solubility in urine, or finally an attempt to increase the volume of urine flow so that at no time does the cystine concentration reach saturation levels. These possibilities have been discussed in some detail by Dent and Senior [*13*].

The most direct method of influencing the quantity of cystine excreted would be by restriction of protein in the diet. Severe restriction of protein intake does, in fact, cause an appreciable reduction in cystine output [*13*]. However severe protein restriction is an undesirable therapy.

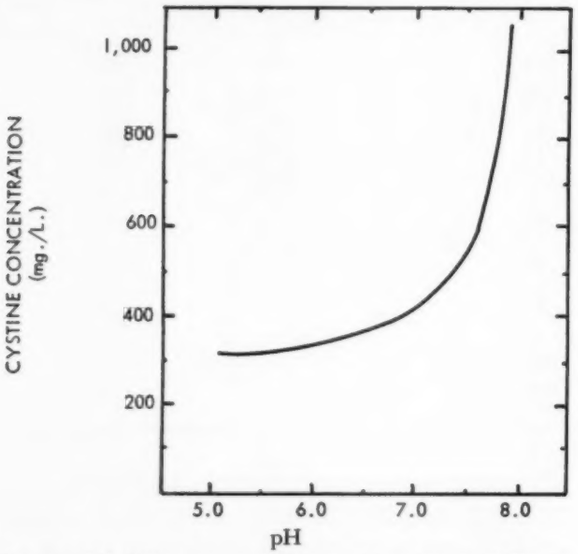


FIG. 6. Solubility curve of cystine in urine at different pH's (after DENT and SENIOR [*13*]).

Lysine is being excreted by these patients in even greater quantities than cystine and this is an essential amino acid. There could be a real danger of going into negative nitrogen balance, particularly if such therapy was applied in growing children. Neither moderate protein restriction nor a great increase in protein intake seem to have a very great effect on cystine output. (Table II.) There seems to be, therefore, no virtue in advising protein restriction.

The use of alkalis in order to render the urine more alkaline and hence increase the solubility of cystine has been widely recommended. The difficulty here lies in the character of the pH solubility curve. (Fig. 6.) The urine pH must evidently be raised to over pH 7.6 before any large increase in cystine solubility occurs. To reach and maintain such a high pH would require the continuous administration of extremely large doses of alkali and this would not appear to be very practical for long periods as a prophylactic measure.

The most hopeful line of attack is to attempt to increase the volume of urine flow [*13*]. It seems that the daily output of cystine could in the average patient be held in solution by about 3 L. of urine at pH 6. The difficulty, of course, arises from the diurnal rhythm. It is fairly easy to maintain a high rate of flow during the day, but special measures are necessary at night. Dent and Senior recommend for routine practice that the patient, besides drinking approximately 3 L. of liquid during the day, should also take at least two glasses of fluid before going to bed and another two glasses when he wakes to empty his bladder at about 2 a.m. in the morning. The best routine can no doubt be worked out individually for each patient. The aim should be to maintain a urine flow of at least 2 ml. per minute for each four-hour period throughout the day and night.

TABLE II
CYSTINE EXCRETION AT DIFFERENT LEVELS OF PROTEIN INTAKE IN A SEVEN YEAR OLD GIRL WITH CYSTINURIA [*13*]

Diet	Protein Content (gm./24 hr.)	Cystine Excretion on Consecutive Days (mg./24 hr.)	Average Cystine Excretion
"Normal"	63.0	539, 488, 572, 555	538
"Vegetarian"	38.5	460, 488, 468, 480, 463, 490	476
Very low protein	20.0	336, 363, 371, 365, 331	353
High protein	117.0	484, 547, 507	513

Even if this approach proves to be useful in preventing stone formation, it would perhaps be too hopeful to expect that it could succeed in dissolving large stones once they are formed. Its value therefore is likely to be mainly prophylactic, either to prevent stones from ever forming, in predisposed persons, or once they have formed to prevent recurrence after surgery. Here the genetical approach is of practical importance. One in four of the brothers and sisters of known cystinuric patients is likely to be similarly affected. If the urines of such persons are examined, it is comparatively easy to pick out the homozygous persons who are predisposed to stone formation but in whom stones may not yet have formed. Proper advice can perhaps prevent them from ever doing so.

REFERENCES

1. ACKERMANN, D. and KUTSCHER, F. Uber das Vorkommen von Lysin in Harn bei Cystinurie. *Ztschr. f. Biol.*, 57: 355, 1912.

2. Arrow, V. and Westall, R. G. Personal communication, 1956.
3. Alsberg, C. L. and Folin, O. Protein metabolism in cystinuria. *Am. J. Physiol.*, 14: 54, 1906.
4. Bach, S. J. The Metabolism of Protein Constituents in the Mammalian Body. Oxford, 1952. Clarendon Press.
5. Beyer, H. K., Wright, L. D., Russo, H. F., Skegg, H. R. and Shoner, G. A. Renal clearance of essential amino acids. Their competition for reabsorption by the renal tubules. *Am. J. Physiol.*, 151: 202, 1947.
6. Brand, E., Cahill, G. F. and Harris, M. M. Metabolism of various sulfur compounds in cystinuria. *Proc. Soc. Exper. Biol. & Med.*, 31: 348, 1247, 1933.
7. Brand, E., Cahill, G. F. and Harris, M. M. The metabolism of cystine, cysteine, methionine and glutathione. *J. Biol. Chem.*, 109: 69, 1935.
8. Dent, C. E. First International Congress Biochemistry. Abstracts of Communications, p. 196, 1949.
9. Dent, C. E. and Rose, G. A. Amino acid metabolism in cystinuria. *Quart. J. Med.*, 20: 205, 1951.
10. Dent, C. E. and Harris, H. Genetics of cystinuria. *Ann. Eugeni. Lond.*, 16: 60, 1951.
11. Dent, C. E., Heathcote, J. G. and Joron, G. E. The pathogenesis of cystinuria. I. Chromatographic and microbiological studies on the metabolism of sulphur containing aminoacids. *J. Clin. Investigation*, 33: 1210, 1954.
12. Dent, C. E., Senior, B. and Walshe, J. M. Pathogenesis of cystinuria. *J. Clin. Investigation*, 33: 1210, 1954.
13. Dent, C. E. and Senior, B. Studies on the treatment of cystinuria. *Brit. J. Urol.*, 27: 317, 1955.
14. Fowler, D. I., Harris, H. and Warren, F. L. Plasma-cystine levels in cystinuria. *Lancet*, 1: 544, 1952.
15. Garrod, A. E. Inborn Errors of Metabolism, 2nd Ed. Oxford, 1923. Oxford University Press.
16. Harris, H. and Warren, F. L. Quantitative studies on urinary cystine in patients with cystine stone formation and their relatives. *Ann. Eugeni. London*, 18: 125, 1953.
17. Harris, H. and Warren, F. L. Excretion of aminoacids in cystinurics. *Biochem. J.*, 57: XXXII, 1954.
18. Harris, H., Mittwoch, U., Robson, E. B. and Warren, F. L. The pattern of aminoacid excretion in cystinuria. *Ann. Human Genetics, London*, 19: 196, 1955.
19. Harris, H., Mittwoch, U., Robson, E. B. and Warren, F. L. Phenotypes and genotypes in cystinuria. *Ann. Human Genetics, London*, 20: 57, 1955.
20. Harris, H. and Robson, E. B. Variation in homozygous cystinuria. *Acta Genetics*, 5: 381, 1955.
21. Hele, T. S. Metabolism in cystinuria. *J. Physiol.*, 39: 52, 1909.
22. Lovney, J. M., Berglund, H. and Graves, R. C. A study of several cases of cystinuria. *J. Biol. Chem.*, 57: 515, 1923.
23. Robson, E. B. and Rose, G. A. The effect of intravenous lysine on the renal clearances of cystine, arginine and ornithine in normal subjects, in patients with cystinuria and Fanconi syndrome and in their relatives. *Clin. Sc.* (In press).
24. Stein, W. H. Excretion of amino acids in cystinuria. *Proc. Soc. Exper. Biol. & Med.*, 78: 705, 1951.
25. Stein, W. H. and Moore, S. The free aminoacids of human blood plasma. *J. Biol. Chem.*, 211: 915, 1954.
26. Yeh, H. L., Frankl, W., Dunn, M. S., Parker, P., Hughes, B. and György, P. Urinary excretion of amino acids by cystinuric subject. *Am. J. M. Sc.*, 214: 507, 1947.
27. Wollaston, W. H. On cystic oxide, a new species of urinary calculus. *Phil. Tr. Roy. Soc.*, p. 223, 1810.

End of Symposium on Inborn Errors of Metabolism

Clinical Studies

Hypertrophy of the Right Ventricular Outflow Tract*

A Concept of the Electrocardiographic Findings in Atrial Septal Defect

S. Gilbert Blount, Jr., M.D., E. Addis Munyan, Jr., M.D. *and* Murray S. Hoffman, M.D.

Denver, Colorado

Because of the growing dissatisfaction with the concept of incomplete right bundle branch block as an explanation for the electrocardiographic findings in atrial septal defect, a study was undertaken to determine the frequency of the rSr′† pattern seen in right precordial leads in normal children and to determine some of the factors that might increase the height of the r′ wave, thereby giving the rSR′ complex with a total QRS time of 0.08 to 0.10 second found in certain congenital and acquired cardiac defects.

Mounsey and associates [*1*] noted the frequency of the rSr′ complex in the right precordial leads in normal healthy men, finding this type of complex in fourteen of thirty men studied. Camerini [*2*] also studied the frequency of the rSr′ complex in multiple right sided chest leads in normal subjects and found the pattern in forty of fifty patients.

It is considered that the rSr′ complex in right precordial leads represents the normal three vectors of electrical depolarization of the ventricles—septum, free wall of right and left ventricles and the outflow tract of the right ventricle (in the region of the crista supraventricularis).

The first two vectors have been traditionally accepted. The "crista" vector has received somewhat less attention and yet has been studied by a number of workers. Lewis and Rothschild [*3*], in their studies in dogs noted that the conus of the right ventricle and the posterobasal portion of the left ventricle were the last portions of the myocardium to be depolarized. Harris [*4*], Sokolow and Friedlander [*5*], and Camerini and Davies [*2*] noted the same general sequential pattern of ventricular depolarization.

Kossman [*6*] discussed the possibility that the rSR′ pattern seen in certain congenital defects might be a derivation of the rSr′ observed normally over the right ventricle with the added effects of hypertrophy of the conus area.

Anatomically, the outflow tract of the right ventricle (or infundibulum) is bounded anteriorly and to the right by free wall of the right ventricle. Posteriorly and to the left lies the interventricular septum behind which is the outflow tract of the left ventricle. The pulmonary valve lies superiorly. A triangular ridge of muscle, the crista supraventricularis, shaped like an inverted V, extends from anterior free wall to interventricular septum. Below the septal insertion of this inverted V, the moderator band extends as a bridge from septum to anterior papillary muscle. (Fig. 1.)

Embryologically, this outflow area is the remnant of the old bulbus cordis, which phylogenetically acted as a regulator of blood flow, protecting the fine gill capillaries from the forceful ejection of blood from the ventricle.

Believing that the rSR′ pattern with QRS time of 0.08 to 0.10 second to be due to hypertrophy of the outflow tract of the right ventricle and not the result of a disturbance in ventricular conduction, it was decided to investigate the matter further.

* From the Heart Station of the Division of Cardiology, Department of Medicine, University of Colorado School of Medicine, Denver, Colorado.

† Lower and upper case letters indicate small or large deflections, respectively, of R, S or R′.

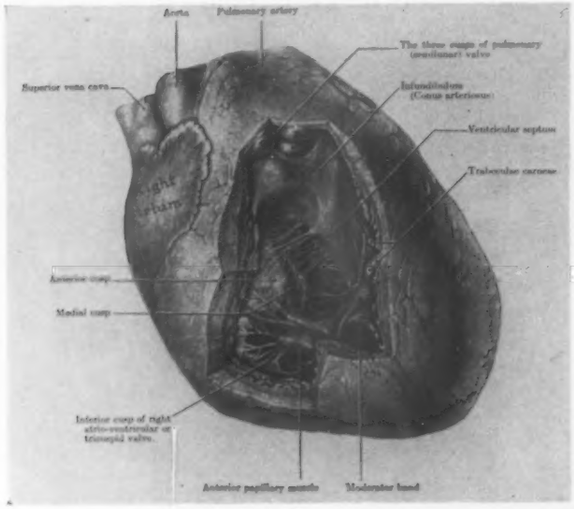


FIG. 1. The interior of the right ventricle showing the infundibulum (including the crista supraventricularis) as an inverted V-shaped structure in the right ventricular outflow tract. The crista extends from septum to anterior wall of right ventricle. (Reproduction from Grant's Atlas of Anatomy).

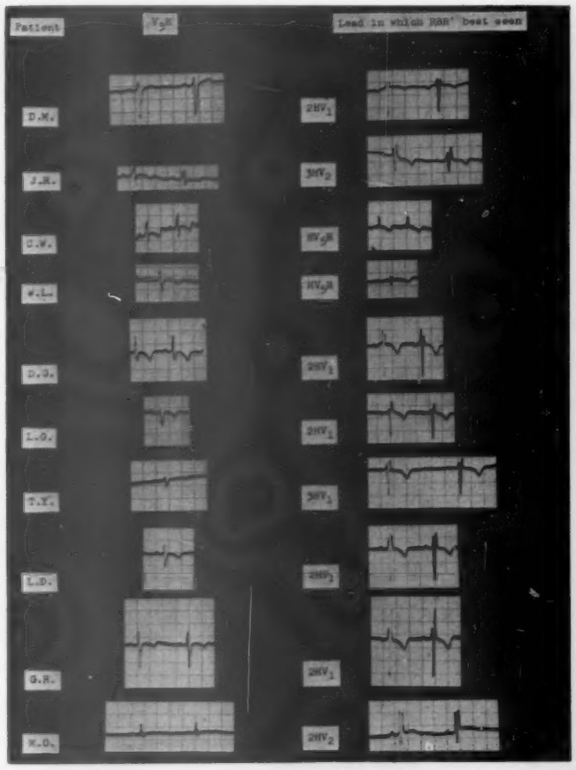


FIG. 2. Types of patterns noted in lead V_5R in fifty normal children (left hand column) together with "crista" pattern obtained in the same child in leads farther to right and higher than traditional electrode placement (right hand column). HV_5R refers to electrode placed one interspace higher than ordinary V_5R; $2HV_5R$ and $3HV_5R$ refers to electrode being placed two or three interspaces higher. The same symbols are used for leads V_1 and V_2.

PRESENT STUDY

As a source of normal hearts, patients were studied who had been referred to the Denver Rheumatic Fever Diagnostic Service at the University of Colorado School of Medicine. These patients, all children, had been referred by private physicians for evaluation of cardiac murmurs. Those who, after evaluation of history, physical examination, fluoroscopy and electrocardiogram, were considered to have functional murmurs and normal hearts were included in the series. Multiple precordial leads were then taken with a one-half inch diameter suction electrode, starting with leads V_6R to V_6 (V_6R, V_5R, V_4R, V_3R, V_1, V_2, V_3, V_4, V_5, V_6). The same leads (V_6R to V_6) were then taken one, two, three or four intercostal spaces above.

Direct electrocardiographic leads were taken at the time of surgery on five patients with atrial septal defects. Multiple points on the epicardial surface of the right ventricle were explored, including (1) a point on the outflow tract of right ventricle just below the pulmonary valve; (2) mid-right ventricle; (3) right ventricular inflow tract; and (4) right ventricle just adjacent to the interventricular septum and left ventricle.

Intracavitary electrocardiograms were recorded on five patients with atrial septal defects. Tracings were obtained during withdrawal of the catheter with special attention to the types of patterns obtained from the pulmonary artery, pulmonary valve area, right ventricular outflow tract, mid-right ventricle and right ventricular inflow tract.

Serial electrocardiographic tracings were observed on eight patients with isolated valvular pulmonic stenosis and fourteen patients with atrial septal defects to observe any serial changes in the abnormal electrocardiograms following the surgical correction.

Also, electrocardiograms of ten patients with tight mitral stenosis and varying degrees of right ventricular hypertrophy were studied to determine the types of patterns and the sequential changes in the electrocardiograms following commissurotomy.

RESULTS

Study of Normal Children. Forty-nine of fifty normal children studied with multiple right

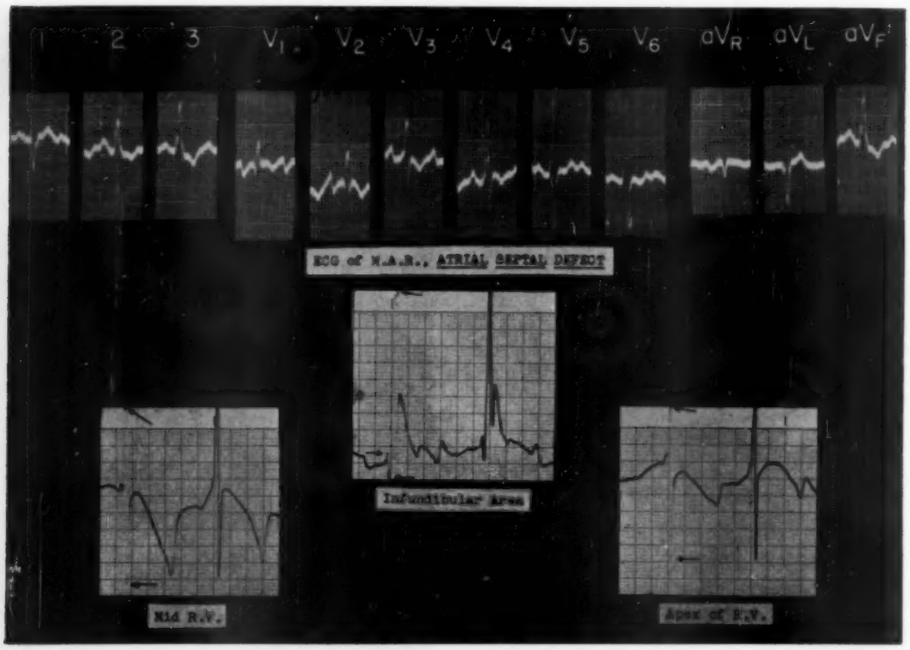


FIG. 3. Direct epicardial leads at surgery demonstrating an rsR′ in the infundibular area just beneath the pulmonary valve and the RS pattern in the mid-right ventricle and at the apex of the right ventricle. Two complexes are shown at each area. One complex is not retouched and arrow points to the limits of the QRS. The other complex has been retouched for clarity.

precordial leads revealed rSr′ patterns in at least one of these right chest leads. This pattern was not observed in V_3R, but rather in a higher right chest lead or one located farther to the right. The configurations noted in V_3R were of an rS pattern or splintered complexes with an embryonic r either on the upstroke or downstroke of the S wave or an rSr′s′ pattern. The splintered patterns were considered as crista variants and transitional between strictly crista patterns seen in higher chest leads farther to the right and right ventricular type complexes seen in V_1 and V_2. (Fig. 2.)

Direct Leads from the Epicardial Surface of Right Ventricle. In all five cases of atrial septal defects studied by this method the rSR′ pattern or rSR′s′ pattern was localized to the area beneath the pulmonary valve. The areas of mid-right ventricle, inflow tract of right ventricle, and mid-right ventricle near the interventricular septum revealed an RS pattern or rS pattern. (Fig. 3.)

Intracavitary Electocardiograms Studied on Withdrawal of the Catheter. In all cases studied a transition zone was observed between the QS or QR pattern of pulmonary artery and the rS pattern of mid-right ventricle. This intermediate pattern, in the region of the right ventricular outflow tract was a splintered complex of the delayed R wave variety (rSR′ or rSR′s′). Often in the same patient, on successive observation during withdrawal of the catheter, an rSr′ pattern was obtained on one withdrawal and an rSR′s′ pattern on another withdrawal indicating the variation of the pattern on slight differences in position of the catheter as it passed the complex structures in the right ventricular outflow tract. The intermediate type complexes appeared as one approached the pulmonary valve on the withdrawal and not necessarily at the moment of passage through the pulmonary valve. (Fig. 4.)

Studies of Eight Patients with Valvular Pulmonic Stenosis Following Surgical Correction. All eight patients studied had definite right ventricular hypertrophy prior to surgery. Following surgical correction, in five of the eight patients the lead V_3R changed to an rsR′ pattern (hypertrophied crista) in ten days to thirty-five months. (Fig. 5.) The remaining three patients in this series continued to show qR or RS patterns although the R wave decreased in magnitude over the follow-up period of thirteen, thirty-four and forty-four months.

Studies of Fourteen Patients with Atrial Septal

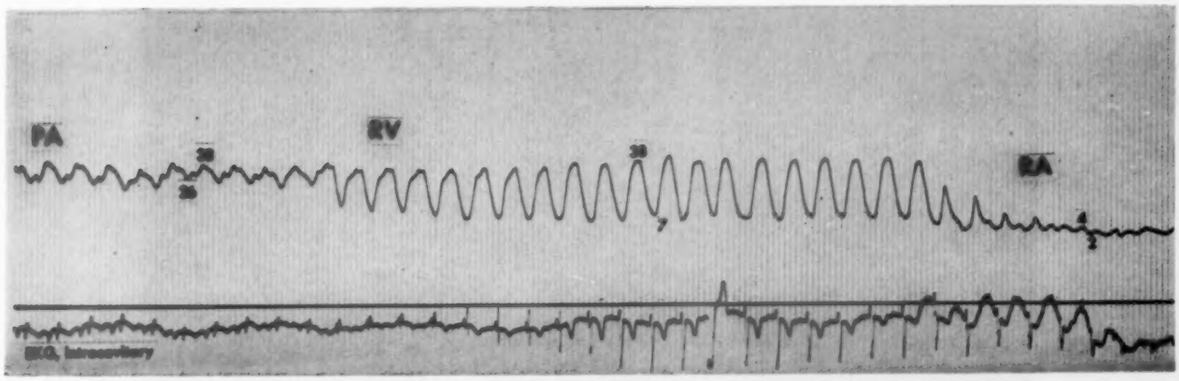


FIG. 4. Intracavitary electrocardiogram obtained on catheter withdrawal. Note the transition zone between the QR pattern in the pulmonary artery and the rS pattern of the mid-right ventricle. This intermediate pattern, in the region of the right ventricular outflow tract is first an rsr′ complex and then an rsR′S′ complex.

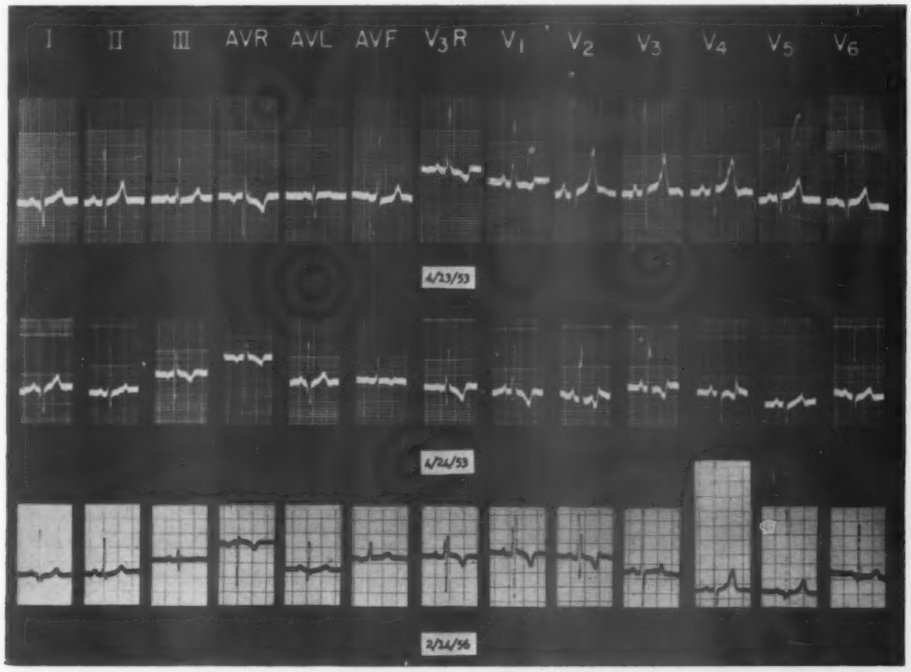


FIG. 5. Patient with pure pulmonic stenosis showing evolutionary stages of the electrocardiogram as the right ventricular hypertrophy regressed. The right precordial leads reveal changes from a notched R pattern to an rSr′ pattern suggesting that the latter is merely a lesser degree of right ventricular hypertrophy.

Defect Following Surgical Correction. Following complete closure of the defect, serial electrocardiograms showed the following: (1) One patient with right ventricular hypertrophy (qR) before surgery continued to have right ventricular hypertrophy, although the R wave postoperatively decreased in magnitude over a follow-up period of nineteen months. (2) In four patients with right ventricular hypertrophy who had an RS pattern before surgery, the leads changed to an rSR′ pattern between five days and fifteen months, but did not change to an rSr′ pattern. (3) In one patient with right ventricular hypertrophy, with an RS configuration before surgery, the lead changed to an rSR′ pattern in four days and to an rSr′ pattern within seven months. (4) Five patients with an rSR′ pattern prior to surgery continued to show the same pattern with a follow-up period of five to twenty-one months, although there was a decrease in the magnitude of the R′ in four of the five cases. (5) Three patients changed from an

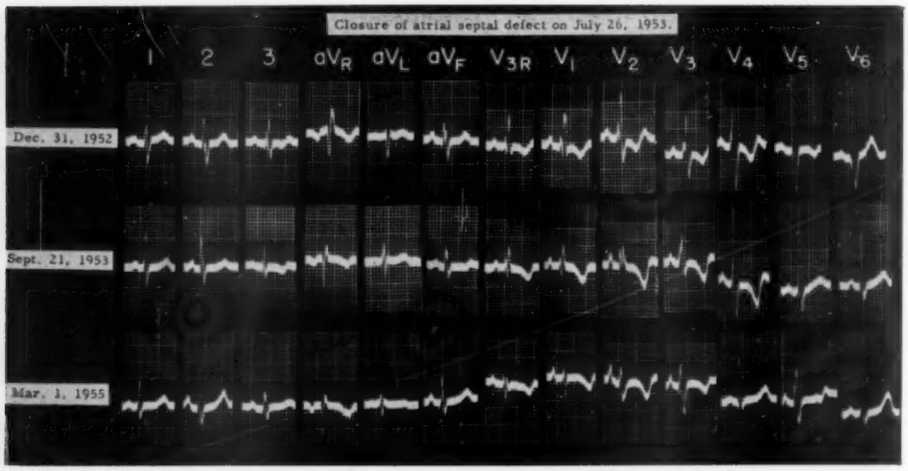


FIG. 6. Change from rsR′ in right precordial leads to rSr′ following closure of atrial septal defect in a six year old boy. The rsR′ is thought to be due to hypertrophied crista whereas the rSr′ is thought to be a normal crista pattern.

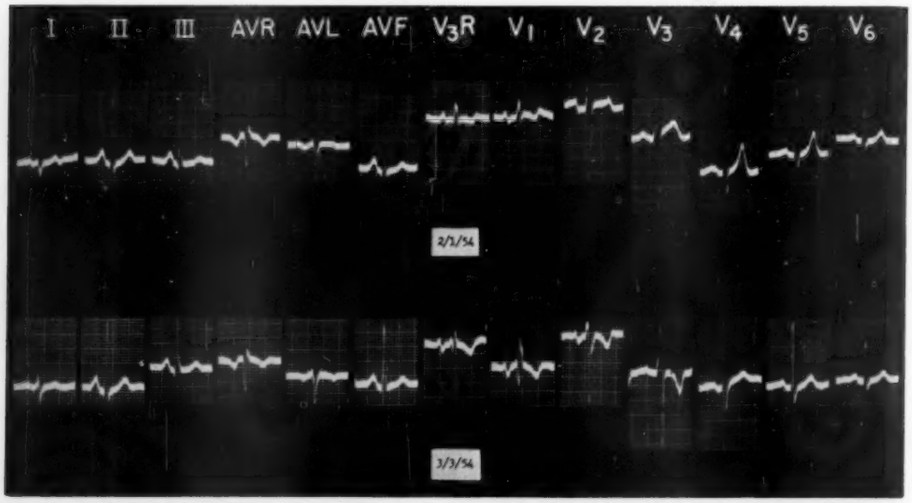


FIG. 7. This patient was a twenty-six year old man who underwent mitral commissurotomy on February 3, 1954. The QRS complex in lead V_3R changed from an Rs to an rSR′ in one month, suggesting the latter to be simply a lesser degree of RVH (outflow tract hypertrophy) rather than an incomplete right bundle branch block.

rSR′ pattern before surgery to an rSr′ pattern between seven and fourteen months postoperatively (Fig. 6.)

Studies of Ten Patients with Mitral Stenosis Following Surgery. Of ten patients who showed electrocardiographic evidence of right ventricular hypertrophy before surgery, four patients exhibited changes in the electrocardiograms from an RS pattern of right ventricular hypertrophy to an rSR′ pattern. (Fig. 7.)

COMMENTS

A characteristic pattern in the electrocardiogram (rSr′) due to the depolarization of the outflow tract of the right ventricle was demonstrated in the right precordial leads in 98 per cent of the patients with normal hearts. This pattern is considered to be the result of three vector forces. The first is due to depolarization of the interventricular septum and portions of the right ventricle resulting in a vector with positivity toward the right and an upright deflection in right precordial leads. The second is due to the simultaneous depolarization of the left and right ventricles with a resultant force vector directed downward and toward the left, causing a downward deflection or S wave. The third is due to the late depolarization of the area of the right

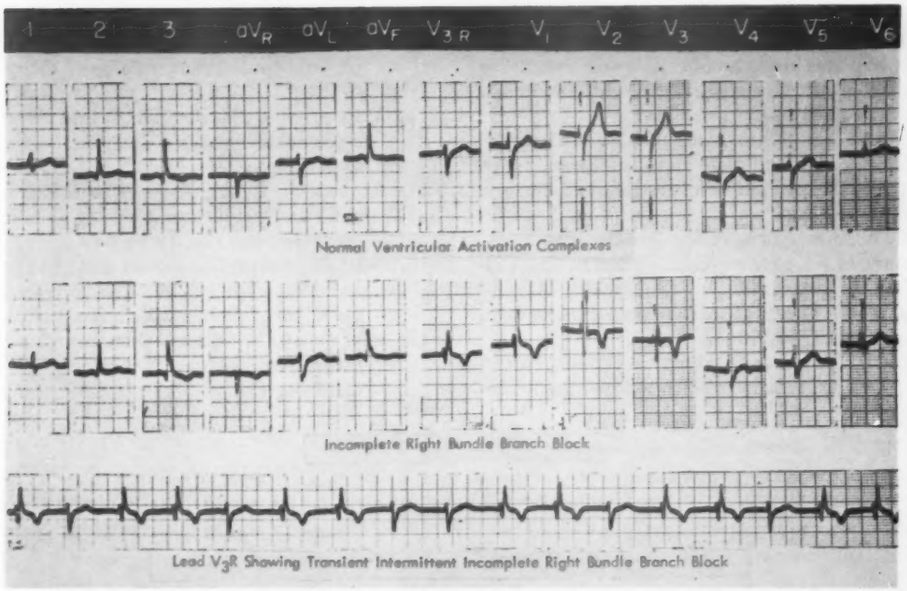

1 2 3 aVR aVL aVF V3R V1 V2 V3 V4 V5 V6
Normal Ventricular Activation Complexes
Incomplete Right Bundle Branch Block
Lead V3R Showing Transient Intermittent Incomplete Right Bundle Branch Block

FIG. 8. This patient was a thirty-two year old man with pneumothorax following rib fracture. Lead V_3R shows rSR′ patterns alternating with rS patterns. The first two electrocardiograms were obtained minutes apart. This must certainly be a conduction disturbance.

ventricular outflow tract with the resultant vector again directed toward the right, causing a terminal positive deflection or r′ wave.

Wilson [7,8] has shown that in experimental animals with right bundle branch block, a characteristic rSR′ pattern was obtained in direct epicardial leads from all areas of the right ventricle—both inflow and outflow tract. These findings were not observed in the cases of atrial septal defect studied as part of the project herein reported. Direct epicardial leads during surgery on patients with atrial septal defects revealed the rSR′ or rSR′s′ patterns only from the right ventricular outflow tract with RS patterns from the mid-right ventricle. This observation correlated well with intracavitary electrocardiograms taken during catheter withdrawal.

The gradual transition of obvious right ventricular hypertrophy patterns in valvular pulmonary stenosis to rSR′ and finally rSr′ patterns following surgical correction further substantiates the concept that hypertrophy of the right ventricular outflow tract is the explanation of these findings. Studies on patients with mitral stenosis and atrial septal defects after surgery were in accord with this concept.

Though it is considered that the rSR′ pattern present in atrial septal defects and other congenital and acquired defects is due to hypertrophy of the right ventricular outflow tract, it is believed that this pattern can be precisely duplicated by incomplete right bundle branch block. Figure 8 shows the electrocardiogram of a thirty-two year old man with spontaneous pneumothorax. The rSR′ pattern in V_1 reverted to normal within a matter of minutes and this certainly cannot be explained on the basis of outflow tract hypertrophy.

SUMMARY

Evidence is presented to support the concept that the rSR′ pattern with a total QRS time of 0.08 to 0.10 seconds observed in certain congenital and acquired lesions—but predominately atrial septal defects—is due to hypertrophy of the right ventricular outflow tract rather than to incomplete right bundle branch block.

REFERENCES

1. MOUNSEY, J. P. D., RITZMAN, L. W. and SELVERSTONE, N. J. Cardiographic studies in severe pulmonary emphysema. *Brit. Heart J.*, 14: 442, 1952.
2. CAMERINI, F. and DAVIES, L. G. Secondary R waves in right chest leads. *Brit. Heart J.*, 17: 28, 1955.
3. LEWIS, T. and ROTHSCHILD, M. A. The excitatory process in the dog's heart. II. The ventricles. *Phil. Tr. Roy. Soc., London*, Series B 206: 181, 1915.
4. HARRIS, A. S. The spread of excitation in turtle, dog, cat and monkey ventricles. *Am. J. Physiol.*, 134: 319, 1941.
5. SOKOLOW, M. and FRIEDLANDER, R. D. The normal unipolar precordial and limb lead electrocardiogram. *Am. Heart J.*, 38: 665, 1949.

6. Kossman, C. E., Berger, A. R., Rader, B., Brumlik, J., Briller, S. A. and Donnelly, J. H. Intracardiac and intravascular potentials resulting from electrical activity of the normal human heart. *Circulation*, 2: 10, 1950.
7. Wilson, F. N. and Herrmann, G. R. An experimental study of incomplete bundle branch block and of the refractory period of the heart of the dog. *Heart*, 8: 229, 1921.
8. Wilson, F. N., Johnston, F. D., Rosenbaum, F. F., Erlanger, H., Kossman, C. E., Hecht, H., Cotrim, N., de Oliveira, R. M., Scari, R. and Barker, P. S. The precordial electrocardiogram. *Am. Heart J.*, 27: 19, 1944.
9. Johnson, J. B., Ferrer, I., West, J. R. and Cournand, A. The relation between electrocardiographic evidence of right ventricular hypertrophy and pulmonary arterial pressure in patients with chronic pulmonary disease. *Circulation*, 1: 536, 1950.
10. Myers, G. B., Klein, H. A. and Stofer, B. E. The electrocardiographic diagnosis of right ventricular hypertrophy. *Am. Heart J.*, 35: 1, 1948.
11. Rosenman, R. H., Pick, A. and Katz, L. N. Intraventricular block: review of the literature. *Arch. Int. Med.*, 86: 196, 1950.
12. Sodi-Pallares, D., Rodriguez, M. I., Chait, L. O. and Zuckermann, R. The activation of the interventricular septum. *Am. Heart J.*, 41: 569, 1951.
13. Walmsley, T. Quain's elements of anatomy. In: The Heart, vol. 14, pt. 3. London, 1929. Longmans, Green & Co.

Systemic Manifestations of Hypernephroma*

A Review of 273 Cases

LAWRENCE BERGER, M.D. *and* MARVIN W. SINKOFF, M.D.
New York, New York

THE diagnosis of hypernephroma, once suspected, usually presents little difficulty. In most patients with hypernephroma fairly classic symptoms or signs develop such as hematuria, flank pain, a palpable flank mass, occasionally a bruit audible by auscultation over the tumor. These patients give unequivocal urographic evidence of renal tumor. However, in a significant number of cases none of the classic local manifestations are present at the onset of the disease. The internist, rather than the urologist, is consulted for elucidation of a bizarre fever, vague anorexia, anemia of unknown origin or an ill defined abdominal mass. Many such cases of occult hypernephroma occur and masquerade under various guises which pose a serious challenge in differential diagnosis. The correct diagnosis may defy detection for prolonged periods of time, yielding only after exhaustive investigation.

It is the cases of hypernephroma that present with atypical or obscure clinical pictures with which we are concerned in this report. It is hoped that by a review of known cases of hypernephroma, some clues can be obtained to create a higher index of suspicion for the diagnosis in the more difficult clinical problems.

Several aspects of this problem have been described previously; the occurrence of fever in hypernephroma [*1–7*] is well known, as is the association of amyloid with this renal tumor [*8–10*]. Reference is made in the literature to the occurrence of polycythemia concomitantly with hypernephroma [*11–14*], and to the occasional case presenting with neurologic, osseous or pulmonary complaints [*15*]. These aspects of hypernephroma, as well as others, will be presented so as to emphasize the various clinical patterns that may develop in this most intriguing of tumors.

MATERIAL

The material for this report is derived from an analysis of 273 cases of hypernephroma studied at The Mount Sinai Hospital in the years 1933–1955. Only those cases have been included in which the pathologic diagnosis was made either on a surgical or postmortem specimen. Table I lists the incidence of the various symptoms and signs that comprise the usual clinical picture of hypernephroma. These data are in accord with previous figures available in the literature [*16–18*].

TABLE I
HYPERNEPHROMA: PERTINENT DIAGNOSTIC DATA IN 273 CASES

	Number	Per cent
Sex:		
Male	178	65
Female	95	35
Age:		
to 29	4	1.5
30 to 39	12	4.5
40 to 49	37	13
50 to 59	112	41
60 and above	108	40
Pain	115	42
Gross hematuria	50	18
Weight loss	85	31
Fever	44	16
Mass	170	63
Anemia*	77	28
Positive urographic findings†	252	100

* Defined as a hemoglobin value below 10 gm. per 100 cc. of blood.

† Findings compatible with renal tumor were present in 252 cases in which urography was recorded in the chart. In twenty-one cases no such record was found.

Of greater interest to us were those cases culled from the aforementioned 273 in which the "non-urologic" aspects were most prominent. In Table II we have summarized our findings in this regard.

* From the Department of Medicine, The Mount Sinai Hospital, New York, N. Y. Dr. Berger was a Trainee of the National Heart Institute during the period 1954–1955, when this report was prepared.

ANALYSIS OF DATA

Fever. In the 273 cases, fever, documented by history and preoperative temperature course, occurred in forty-four or 15 per cent of the patients. In thirty-seven of these cases the diagnosis of hypernephroma was made quite readily

TABLE II
DIAGNOSTIC DIFFICULTIES IN HYPERNEPHROMA: DATA ON 273 CASES

	Number	Per cent
Fever of undetermined origin	7	2.5
Amyloidosis	8	3.0
Absence of hematuria	120	44.0
Signs of debility as sole manifestation	5	1.8
Polycythemia	5	1.8
Misdiagnosed abdominal mass	10	3.6
Metastasis 14 years after nephrectomy for original tumor	1	0.4
Presenting complaint pulmonary	10	3.6
Presenting complaint osseous	8	3.0
Presenting complaint neurologic	5	1.8

because of associated findings. However, seven patients (2.5 per cent) were admitted to the medical service with "fever of undetermined origin," with fever and malaise as the cardinal complaints.

The nature of the fever varied. Four patients exhibited a low grade fever (below 102°F.); the remaining three had daily elevations as high as 105°F. The duration of the febrile course varied from two to six months. The diagnosis of hypernephroma eventually was suspected in all seven cases, the most definitive evidence deriving from urographic studies carried out in the course of an exhaustive diagnostic study. Six patients underwent surgery with confirmation of the diagnosis. In the seventh case diagnosis was confirmed at the autopsy table, since the patient's clinical condition precluded surgical intervention.

Amyloid. This series contained eight cases (2.9 per cent) of amyloid, present either in the resected kidney which was the site of the hypernephroma (four cases) or throughout the body in the distribution commonly referred to as characteristic of "secondary" amyloidosis (four cases). Of these eight cases five have been reported from this hospital previously [*8,9*].

No statement can be made as to the extent of amyloid involvement in those instances where only the excised kidney was available for examination. Also, no information is available from the hospital record as to the subsequent course of the amyloid, following removal of the renal tumor.

Of interest is the fact that in only one of the eight cases was the diagnosis of amyloid clinically suggested. No suspicion was entertained in seven cases. Indeed, two of these latter presented as "fever of undetermined origin," rather than as easily identifiable hypernephroma. Although several patients had some proteinuria, the degree was not sufficient to implicate amyloid involvement of the kidney.

Hematuria. On history, the symptoms of gross hematuria occurred in fifty patients (18 per cent). However, a small portion of these did not reveal hematuria even by microscopic examination at the time of admission to the hospital. In the total series of 273 cases urine analysis revealed that forty-eight, or 18 per cent, had marked hematuria on admission; thirty-one, or 11 per cent, had moderate hematuria; and seventy-four, or 27 per cent, had microscopic hematuria of at least an occasional red blood cell per high power field. Thus 120 cases, or 44 per cent, gave no evidence for genitourinary tract bleeding on urine analysis at the time of hospitalization. Fortunately, the absence of hematuria offered no diagnostic difficulty in itself because of the presence of other symptoms and signs of hypernephroma.

Signs of Debility. At least half of the patients on admission to the hospital presented a picture which included such complaints as weight loss, weakness, anorexia and anemia. In general, these were part of the picture of a chronic disease process and, together with other associated findings, led eventually to the diagnosis of renal tumor. Such symptoms were of little diagnostic value to the examining physician, but they did not becloud the diagnosis because of the presence of localizing findings. However, five patients (1.8 per cent) sought medical advice solely because of these complaints: weakness, weight loss, anorexia. Moreover, clinical examination failed to reveal any readily discernible cause for these signs of debility. Such patients therefore were subjected to energetic investigations involving biochemical, hematologic and radiologic studies. Only then was the diagnosis of a renal neoplasm made, on the basis of abnormal urographic findings.

Misdiagnosed Abdominal Mass. An upper abdominal mass was palpable in 170 cases (62 per cent). Of these, fully 50 per cent (eighty-five) were painless. Patients with masses not suspected to be renal in origin numbered ten, or about 4 per cent. Most often these latter patients presented with weight loss, anorexia, weakness and anemia. The mass, if felt in the right upper abdomen, was variously interpreted as liver, gall bladder or colon; in the left upper abdomen, the organs erroneously implicated were colon, spleen and, to a lesser extent, pancreas and stomach. Apparently, the usual criteria of physical diagnosis were found wanting, in these ten cases, to identify properly the nature of the palpable mass. However, appropriate radiologic studies fulfilled this purpose. All ten patients underwent laparotomy, with final confirmation of the diagnosis.

Late Metastasis. The occurrence of metastases many years after nephrectomy for hypernephroma is well known. A striking example is cited.

Patient I. G. (M.S.H. No. 49202), a seventy-two year old white man, in 1941 had a right nephrectomy for a hypernephroma which in the pathologic specimen exhibited invasion of the renal vein. In 1952 because of fever, cough and right chest pain, he was seen again at the hospital, where a right lower lung field infiltrate was found on chest x-ray. Over a period of about four weeks this infiltrate almost completely resolved and the patient was discharged improved, with the presumptive diagnosis of pulmonary infarction. In 1955 he returned with cough and fever of about one month's duration. Again a right lower lung field infiltrate was seen. Bronchoscopy was performed and biopsy yielded bronchial mucosal fragments infiltrated by metastatic hypernephroma, histologically similar to the original specimen of 1941. The patient's subsequent course was a downhill one, and he died three months after admission. Postmortem examination confirmed the presence of metastatic hypernephroma involving the right lower lobe of the lung and right hilar lymph nodes, with infiltration into the esophagus.

This case of late metastasis in hypernephroma demonstrates two aspects worth emphasizing: (1) the difficulty of making such a diagnosis in some instances and, (2) the value of bronchoscopic examination and biopsy in making a diagnosis of metastatic pulmonary disease when the metastasis is endobronchial.

Polycythemia. In addition to the diagnosis of hypernephroma, a clinical diagnosis of polycythemia vera was made in five cases (1.8 per cent), on the basis of hemoglobin values above 17 gm. per cent, red cell counts beyond 6.5 million per cu. mm. and hematocrits of 55 per cent and above.

Furthermore, examination of the hemoglobin levels of all 273 patients revealed fifteen cases (including the aforementioned five), or 5.5 per cent, with levels of 15.0 gm. per cent or more. Such statistics suggest more careful investigation in the future of patients with hypernephroma who may have an associated erythremia.

A Clinical Picture Dominated by Metastatic Organ Involvement (Lungs, Bone and Central Nervous System). Ten patients presented either with cough or evidence of a lung mass on x-ray. In eight instances a history of bone pain was elicited as the chief complaint. Subsequent investigation revealed involvement of the spine in two cases, the humerus in two, the femur in one, and the scapula in one. Roentgenographic examination failed to reveal evidence of metastatic bone involvement in the remaining two cases. Five patients had presenting complaints referable to central nervous system disease, three were affected with an organic mental syndrome, and one each with hemiplegia and paraplegia.

All of these cases, presumed to be metastatic carcinoma, yielded the diagnosis of hypernephroma on appropriate investigation.

COMMENTS

The clinical observations presented in this report demonstrate challenging problems to the internist. Hypernephroma may present with systemic manifestations such as fever, amyloidosis, polycythemia or signs of debility. Difficulties may arise in interpretation of certain localizing signs, for example, the frequent complete absence of even microscopic hematuria, and the occasional errors made in identification of a palpable mass on physical examination. The frequency with which these conditions are met underlines the importance of awareness in the diagnosis of hypernephroma in its protean manifestations.

The occurrence of fever with hypernephroma was first described by Stetter [*19*] in 1887; his case was that of a patient with a fever of 102°F. or more over a two-month period prior to removal of a renal carcinoma. Since then reports of many such cases have appeared, some of

which are listed in the references [*1–7*]. Indeed, the incidence of fever in hypernephroma has been reported as high as 56 per cent [*4*] but generally is stated to range about 20 per cent [*7*]. With better control of infectious diseases, more attention must be paid to chronic granulomatous and neoplastic diseases as causes for unexplained fever [*5*]. Hamman and Wainwright [*2,3*], in a classic discussion of obscure fever, collected ninety-one cases, seven of which were attributable to hypernephroma; two patients had low grade fever, five had high fever. This incidence is to be compared with our four cases of low grade fever and three in which the patients had temperature elevations above 102°F.

In cases of fever associated with hypernephroma it is anticipated that removal of the implicated tumor will eliminate the fever. Persistence or recrudescence of pyrexia after tumor removal may therefore indicate tumor recurrence [*6*].

Hypernephroma is, of course, not the sole neoplasm with a potentiality for producing fever. Incidences of fever of 52 per cent have been cited in uncomplicated tumors of the liver and biliary tree, and 34 per cent in neoplasms of the stomach. Fever has been reported in association with carcinoma of the esophagus, colon, breast and uterus, among other sites [*20*]. The mechanism for this phenomenon is not known, although factors of necrosis and proliferation have been invoked [*21,22*]. Certainly, hypernephroma is marked in its growth by both types of pathologic process, but fever may occur in the absence of extensive necrosis of the tumor or adjacent tissues.

The association of amyloid and hypernephroma is not uncommon, as illustrated by eight cases in this series of 273. Occasionally, fever is present in such cases (two of the eight cases of amyloid in this series presented with fever as one of the manifestations of disease). As has been emphasized in the past [*8,10*], the appearance of proteinuria, renal insufficiency and/or hepatosplenomegaly in a known case of hypernephroma should focus suspicion on the possibility of associated amyloidosis.

Of greater difficulty is the case in which even the primary tumor is not evident, let alone the amyloidosis. This problem was presented by Hyman and Leiter [*8*] in regard to one of their patients, who presented with unexplained fever and was eventually discovered, at autopsy, to have a hypernephroma with amyloidosis of the "secondary" type.

Knowledge concerning the mechanisms whereby primary tumors stimulate the deposition of amyloid is rudimentary. Many factors are thought to contribute. Those given some greater weight are tissue necrosis, chronicity and infection [*8,10,23*]. In 1930 a report by Higuchi [*24*] described a 2.4 per cent incidence of carcinoma in association with amyloidosis. Moschcowitz [*23*] and Dixon [*25*] more recently state that as many as 10 per cent of cases of "secondary" amyloidosis are associated with carcinoma. With improving control of suppurative disease and present day availability of antibiotic therapy, one may expect an even greater proportion of cases of secondary amyloidosis to be associated with carcinoma.

The fate of amyloid already deposited, after removal of the hypernephroma, is not known. The suggestion has been advanced that, as with reported recession of amyloid after elimination of a focus of chronic infection [*26*], a similar phenomenon might occur with successful removal of a hypernephroma [*8*]. The difficulty in evaluating such a possibility lies in the inability to obtain an accurate estimate *in vivo* of the precise extent of the amyloid involvement. Moreover, remaining or recurring tumor in patients with resected hypernephroma and amyloid may contribute to persistence or extension of the amyloid process.

The frequency with which even microscopic hematuria is absent, in our series, corresponds with the experience of others [*16,17*] and reflects the delay in invasion of the renal outflow tract by well contained tumor masses. Thus the diagnosis of hypernephroma should be made independently of the urinary findings.

The single case of a late metastasis presented herein points to the difficulty in diagnosing such cases. Late metastases in hypernephroma are well known to occur [*17,27*]. Of great clinical interest in our case report are the location of the pulmonary metastasis, endobronchially, and the manner of diagnosis by bronchoscopy. This method of diagnosis has been previously reported useful in the presence of pulmonary metastases [*28*].

Only recently is an understanding developing of the cause of anemia in hypernephroma and other malignancies, when blood loss is not prominent. Recent studies indicate the presence of hemolytic factors in many cases. Abnormali-

ties of iron metabolism and bone marrow replacement by metastatic tumor tissue may not be too significant in the development of anemia in cancer [*29*].

The concurrence of hypernephroma and polycythemia has been reported by various investigators [*11–14*]; indeed, disappearance of polycythemia after removal of the hypernephroma has been described [*11,14*]. Recurrence of the erythremia may be interpreted as an indication of tumor recurrence.

If one accepts Wintrobe's reported incidence of polycythemia in a hospital population (0.06 per cent) [*30*], then the number of cases of polycythemia and hypernephroma in our series (five patients, or 1.8 per cent) assumes significance. Polycythemia has been reported to occur in association with other neoplastic processes as well: cerebellar tumor [*30*], uterine fibroids [*31*] and multiple myeloma [*12*]. Etiologic suggestions without experimental foundation have been offered, relating to hemopoietic system stimulation by the tumor or one of its products, or by some indirect, unknown mechanism of adrenocortical stimulation [*11*].

In an excellent discussion of hypernephroma, Creevy [*15*] in 1935 stated that 40 per cent of ninety-two cases were originally misdiagnosed as the following: bone tumor, pulmonary disease, central nervous system disease, cirrhosis, gastric neoplasm, intestinal obstruction. His description of the diagnostic problems in hypernephroma is as appropriate now as when he originally set it forth: "The key to the whole problem lies in considering the *possibility* of a malignant renal tumor in every case of an obscure ailment, particularly in cases of tumors of bone, of unusual lesions of the lungs, gastrointestinal tract and nervous system, and of obscure fever or anemia."

SUMMARY

1. Two hundred seventy-three cases of histologically proven hypernephroma have been reviewed with respect to systemic manifestations and the difficulties that consequently may arise in diagnosis of this tumor.

2. There was a 65 per cent incidence in males, 81 per cent incidence beyond the age of fifty; 42 per cent of the patients had flank pain, 18 per cent gave a history of gross hematuria, 31 per cent of weight loss; 16 per cent of the patients had fever, 63 per cent had a palpable mass, 23 per cent had anemia. Urography revealed findings of renal tumor in every case in which it was performed.

3. The categories of diagnostic difficulties and systemic manifestations presented in this report, with the incidence of each type, were as follows: fever of undetermined origin 2.5 per cent; amyloidosis 2.9 per cent; absence of hematuria 44 per cent; signs of debility as the sole manifestation of hypernephroma 1.8 per cent; polycythemia associated with hypernephroma 1.8 per cent; misdiagnosed abdominal mass temporarily obscuring the diagnosis 4 per cent; late metastasis after removal of hypernephroma 0.4 per cent; primary complaint of a pulmonary, osseous or neurologic nature 9 per cent.

4. The data presented are discussed and the pertinent literature is reviewed. The need for greater suspicion of the diagnosis of hypernephroma in cases with obscure symptoms is emphasized.

REFERENCES

1. Creevy, C. D. Pyrexia in malignant nephroma (hypernephroma). *J. A. M. A.*, 92: 1256, 1929.
2. Hamman, L. and Wainwright, C. W. The diagnosis of obscure fever. I. Diagnosis of unexplained long continued low grade fever. *Bull. Johns Hopkins Hosp.*, 58: 109, 1936.
3. Hamman, L. and Wainwright, C. W. The diagnosis of obscure fever. II. Diagnosis of unexplained high fever. *Bull. Johns Hopkins Hosp.*, 58: 307, 1936.
4. McCague, E. J. Fever as the initial symptom of hypernephroid tumor of the kidney. *Arch. Surg.*, 41: 385, 1940.
5. Beeson, P. B. Fever of obscure origin. *V. A. Tech. Bull.*, vol. 5, series 10, no. 10–72, 1951.
6. Clarke, B. G. and Goade, W. J., Jr. Fever and anemia in renal cancer. *New England J. Med.*, 254: 107, 1956.
7. Keefer, C. S. and Leard, S. E. Prolonged and Perplexing Fevers. Boston, 1955. Little, Brown & Co.
8. Hyman, A. and Leiter, H. E. The association of hypernephroma with amyloidosis of the kidney. *J. Urol.*, 56: 303, 1946.
9. Ellenberg, M. Amyloidosis secondary to malignant Grawitz tumor (hypernephroma) of kidney. *J. Mt. Sinai Hosp.*, 10: 323, 1943.
10. Ask-Upmark, E. On amyloidosis induced by tumors of the kidney. *Acta med. Scandinav.*, 104: 512, 1940.
11. Videbaek, A. Polycythemia vera coexisting with malignant tumors (particularly hypernephroma). *Acta med. Scandinav.*, 138: 239, 1950.
12. Lawrence, J. H. Polycythemia. Physiology, Diagnosis and Treatment Based on 303 Cases. New York, 1955. Grune & Stratton.
13. Gros, H. Hypernephroma and polycythemia—abstract. *J. A. M. A.*, 158: 1476, 1955.
14. Forsell, J. Polycythemia and hypernephroma. *Acta med. Scandinav.*, 150: 155, 1954.
15. Creevy, C. D. Confusing clinical manifestations of

malignant renal neoplasms. *Arch. Int. Med.*, 55: 895, 1935.

16. Soloway, H. Renal tumors. A review of 130 cases. *J. Urol.*, 40: 477, 1938.
17. Melicow, M. M. Classification of renal neoplasms: a clinical and pathological study based on 199 cases. *J. Urol.*, 51: 333, 1944.
18. Abeshouse, B. S. and Weinberg, T. Malignant renal neoplasms. A clinical and pathological study. *Arch. Surg.*, 46: 50, 1945.
19. Stetter. Cited by Keefer, C. S. and Leard, S. E. Prolonged and Perplexing Fevers. Boston, 1955. Little, Brown & Co.
20. Briggs, L. H. The occurrence of fever in malignant disease. *Am. J. Med. Sc.*, 166: 846, 1923.
21. Bennet, I. L., Jr. and Beeson, P. B. Studies on the pathogenesis of fever. I. Effect of injection of extracts and suspensions of uninfected rabbit tissues upon the body temperature of normal rabbits. *J. Exper. Med.*, 98: 477, 1953.
22. Beeson, P. B. In: Harrison, T. R. Principles of Internal Medicine. New York, 1954. Blakiston Co.
23. Moschcowitz, E. Clinical aspects of amyloidosis. *Ann. Int. Med.*, 10: 73, 1936.
24. Higuchi, K. Die Beziehung der Amyloidablagerung zur Vaskularisation in der Milz. *Virchow's Arch.*, 279: 538, 1930.
25. Dixon, H. M. Renal amyloidosis in relation to renal insufficiency. *Am. J. M. Sc.*, 187: 401, 1934.
26. Reimann, H. A. Recovery from amyloidosis. *J. A. M. A.*, 104: 1070, 1935.
27. Graves, R. C. and Mabrey, R. E. Adenocarcinoma of kidney recurrent after 20 years. *New England J. Med.*, 212: 416, 1935.
28. Vinson, P. P. and Martin, W. J. Pulmonary metastasis from hypernephroma diagnosed by bronchoscopy. *Arch. Otolaryngol.*, 15: 368, 1932.
29. Hyman, G. A. and Harvey, J. E. The pathogenesis of anemia in patients with carcinoma. *Am. J. Med.*, 19: 350, 1955.
30. Wintrobe, M. M. Textbook of Clinical Hematology. Philadelphia, 1951. Lea & Febiger.
31. Engel, H. W. and Singer, K. Polycythemia with fibroids. *J. A. M. A.*, 159: 190, 1955.

Seminar on Bone Disease

Osteoporosis*

FREDERIC C. BARTTER, M.D.
Bethesda, Maryland

OSTEOPOROSIS may be defined as that "metabolic" bone disease in which the total body mass of bone is less than that of a normally active subject of comparable size as a result of failure of new bone formation. The qualifications in this definition may be better understood after a brief consideration of normal bone physiology.

The normal bone mass results from a "steady state" in which the rate of bone destruction equals that of bone formation.† Bone formation consists of two processes, formation of bone matrix and the deposition therein of apatite; normally these occur almost simultaneously. Bone destruction is a single process, and "decalcification" of bone does not occur.

"Metabolic" bone diseases are herein defined [*1*] as those in which all the bone tissue is affected to a greater or lesser degree, and in which hormonal factors may play a part in pathogenesis.‡ They include osteomalacia, in which the fundamental disorder is a failure of apatite to be deposited normally in bone matrix, osteitis fibrosa cystica,§ in which the fundamental disorder is an abnormally rapid rate of bone destruction, and osteoporosis. In osteomalacia, the concentration of calcium and phosphate ions in the fluid surrounding bone matrix is clearly of fundamental importance. In osteitis fibrosa, parathyroid hormone may play a part in the bone destruction, and renal failure may contribute thereto in a manner not yet understood. For the present discussion, an understanding of these disorders is of importance in the differential diagnosis of osteoporosis. In contrast to them, osteoporosis is characterized by normal concentrations of circulating calcium and phosphate ions and a normal or, indeed, decreased rate of bone destruction. We are here concerned, then, principally with the factors controlling bone matrix formation.

In postfetal life bone matrix is deposited by osteoblasts in apposition to pre-existing bone or calcified cartilage. It is clear that the strains to which the skeleton is subjected constitute an important stimulus to normal osteoblastic activity and that, other things being equal, the newly formed bone is deposited in a structural pattern ideally designed to meet these strains [*2*]. Because of this, the bone mass is "normally" greater in a heavy person performing physical labor than it is in a light and sedentary one, and the latter may be said to show a degree of osteoporosis.

ETIOLOGY OF OSTEOPOROSIS

Although the pathologic physiology of osteoporosis is incompletely understood, the majority of cases are attributable to inadequacy of osteoblastic activity or of the supply or conservation of nitrogenous materials necessary for bone matrix formation.

In addition to strains on the skeleton, estrogens may stimulate osteoblastic activity under some circumstances [*3,4*]. Diminution in one of these two known stimuli is of paramount importance in the etiology of most cases of osteoporosis; a decrease in activity leads to the osteoporosis of disuse, a decrease in ovarian function leads to the osteoporosis of the postmenopausal state. When limitation of activity is confined to a portion of the skeleton, as with nerve damage [*5*]

† During growth there is obviously a disequilibrium in favor of matrix formation. However, it is apparent that continued bone destruction in this period is a *sine qua non* of bone remodeling.

‡ Osteoporosis provides us with the single exception to this generalization, in that it may be localized, *vide seq.*

§ More correctly, the term osteitis fibrosa cystica *generalisata* is used to define this disorder when it is a metabolic bone disease, whereas the more general term osteitis fibrosa cystica includes cases in which an apparently identical histologic picture is seen in localized areas, surrounded by normal bone.

* From the Section on Clinical Endocrinology, Clinic of General Medicine and Experimental Therapeutics, National Heart Institute, Bethesda, Maryland.

or the application of a cast, osteoporosis may be localized.

In a large group of conditions osteoporosis appears to result from a deficiency in the nitrogenous components of bone matrix, or in their "wasting" to supply energy needs. Deficiency of nitrogenous components of bone matrix may result from simple starvation and some (but not all) cases of so-called "hunger osteopathy" represent osteoporosis. It may result from a deficiency of nitrogen-retaining hormones, as in male eunuchoidism and in senility (where other factors doubtless play a part), or from an excess of steroids inducing glyconeogenesis from protein, as in Cushing's syndrome [*6*] or during therapy with ACTH or cortisone and analogous steroids.

In uncontrolled diabetes [*1*] and in hyperthyroidism [*7*] osteoporosis may occur as the nitrogenous materials essential for bone matrix formation are deaminated and degraded to serve as sources of energy. The signs of osteoporosis may indeed be the presenting ones in obscure cases of thyrotoxicosis. Although osteoporosis is probably the commonest form of generalized bone disease in hyperthyroidism, in some cases areas of bone destruction and rapid bone matrix formation suggestive of osteitis fibrosa have been observed [*7*], and in some rapid bone formation, as estimated from isotope studies, has been noted [*8*]. Combined biochemical and histologic data from a series of well-studied cases are needed to explain fully the varied findings in the bones in this condition.

In hypovitaminosis C formation of bone matrix is defective in common with that of other tissues requiring cement substances, and the bone disease is essentially osteoporosis.

In a small percentage of patients with osteoporosis no known contributing factor can be discerned. These include fragilitas ossium, acromegaly, and those cases designated "idiopathic." Fragilitas ossium is essentially a form of congenital osteoporosis. The abnormality in bone matrix appears in many cases to be part of a generalized disorder of tissue of mesenchymal origin [*9*], which also affects the scleras, tendons and blood vessels. In acromegaly, osteoporosis is a common complication. In many respects it resembles that of the postmenopausal state [*10*], and may be related thereto. It has been suggested [*1*] that the osteoporosis of acromegaly may result from mild hyperadrenocorticism, or hyperthyroidism, resulting from the overproduction of the appropriate tropic hormone by the eosinophilic adenoma; or it may result from the action of growth hormone itself. In the last category of idiopathic osteoporosis, two types of patient are frequently found. In male patients with the disorder there is frequently a history of trauma. Although this suggests the sequence of events to be described, in which a vicious circle is set up, the trauma is often of so mild a degree as not to have required extensive immobilization in the first place. In female patients with idiopathic osteoporosis the bone disorder is often found to have followed an apparently normal pregnancy. In patients of both sexes the serum protein concentrations are low in a surprising number of cases. As discussed later, the diagnosis of idiopathic osteoporosis is often difficult and may ultimately require the demonstration that certain forms of treatment which are effective regularly in the other types are wholly without effect in this one.

DIAGNOSIS OF OSTEOPOROSIS

The clinical diagnosis of osteoporosis rests upon radiologic, chemical and ultimately histologic grounds. It consists essentially in the demonstration of a decrease in the total bone mass without acalcification of new-formed matrix or evidence of bone destruction. Whereas a direct estimate of the bone mass may be made only in the experimental animal, a reasonably certain diagnosis may be established clinically upon indirect evidence.

Bone pain (chiefly in the back) and pathologic fractures (chiefly of the spine and femoral necks) are the commonest presenting symptoms of osteoporosis. Rarely, a renal calculus may provide the first evidence that bone disease is present. Bone tenderness may be demonstrable upon physical examination. Other physical evidence may suggest the nature of the bone disease, as, for example, atrophy and loss of elasticity of skin, and the appearance of senility. Severe osteoporosis may, on the contrary, occur in the absence of any other suggestion of tissue atrophy.

The demonstration of radiolucency of the skeleton by x-ray is of course essential for the diagnosis of osteoporosis. In the absence of objective criteria of bone density, which are seldom available [*11*], the degree of radiolucency is estimated by comparing the shadow of bone with that of soft tissues on the same plate. The

common radiologic practice of describing as "osteoporotic" all radiolucent bone not showing tumor or cysts has indeed some historical justification but it has done much to obscure the understanding of metabolic bone disease. Three radiologic features may in fact strongly suggest a diagnosis of osteoporosis in contradistinction to the other metabolic bone diseases. First, the finding of radiolucency of the spine and pelvis in the presence of a normal skull provides strong evidence in favor of a diagnosis of osteoporosis. So rarely does the skull show significant changes in osteoporosis, however severe, that its involvement strongly suggests another disorder, such as metastatic malignancy. Second, a normal lamina dura about the teeth is regularly present in osteoporosis and regularly absent in the other metabolic bone diseases. The presence of any distinct lamina is of importance; even when nine-tenths of it is lost as a result of "localized" dental pathology, the remainder may furnish clear evidence that osteomalacia or osteitis fibrosa are not present. Third, there are occasionally seen by x-ray vertically arranged "columns" within the vertebral bodies, apparently as a result of selective loss of trabeculae of lesser structural importance. (Fig. 1.) Although a similar picture may (rarely) be seen in osteitis fibrosa, the finding allows a presumptive diagnosis of osteoporosis. Before closure of the epiphyses, a fourth item may be available for the differential diagnosis of radiolucent bone disease by x-ray. Osteoporosis is without effect on the epiphyseal lines, which show well-documented changes in rickets and in renal osteitis fibrosa cystica.

Biochemically, the diagnosis of osteoporosis is established primarily by exclusion of other metabolic bone diseases. As it is primarily a disease of bone matrix, and not of calcium and phosphorus metabolism *per se*, it is the only metabolic bone disease in which normal serum calcium and phosphorus values are found. The one exception to this generalization is seen but rarely; during the rapid onset or aggravation of the disease, the serum calcium may be elevated. This is especially likely to occur in subjects in whom bone formation and destruction had been proceeding rapidly, as in the case of children [*12*] or patients with Paget's disease of bone [*13*]. The urinary calcium may likewise be elevated while the disease is progressing, becoming normal or even low as a new "steady state" is reached wherein the rate of bone destruction keeps pace with a greatly diminished rate of bone formation.

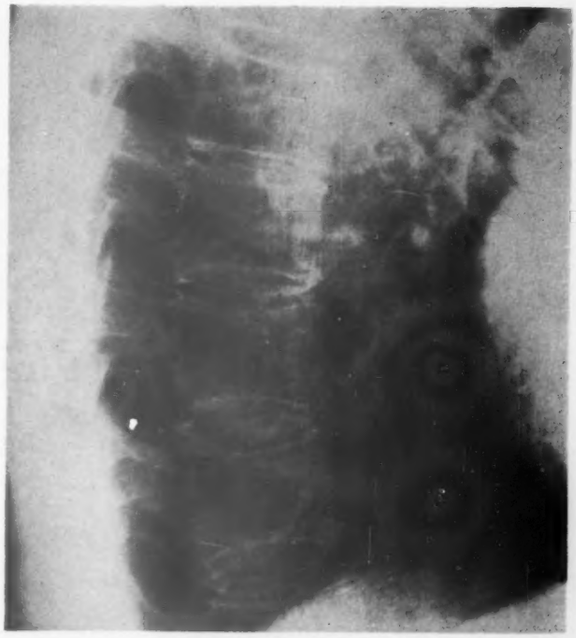
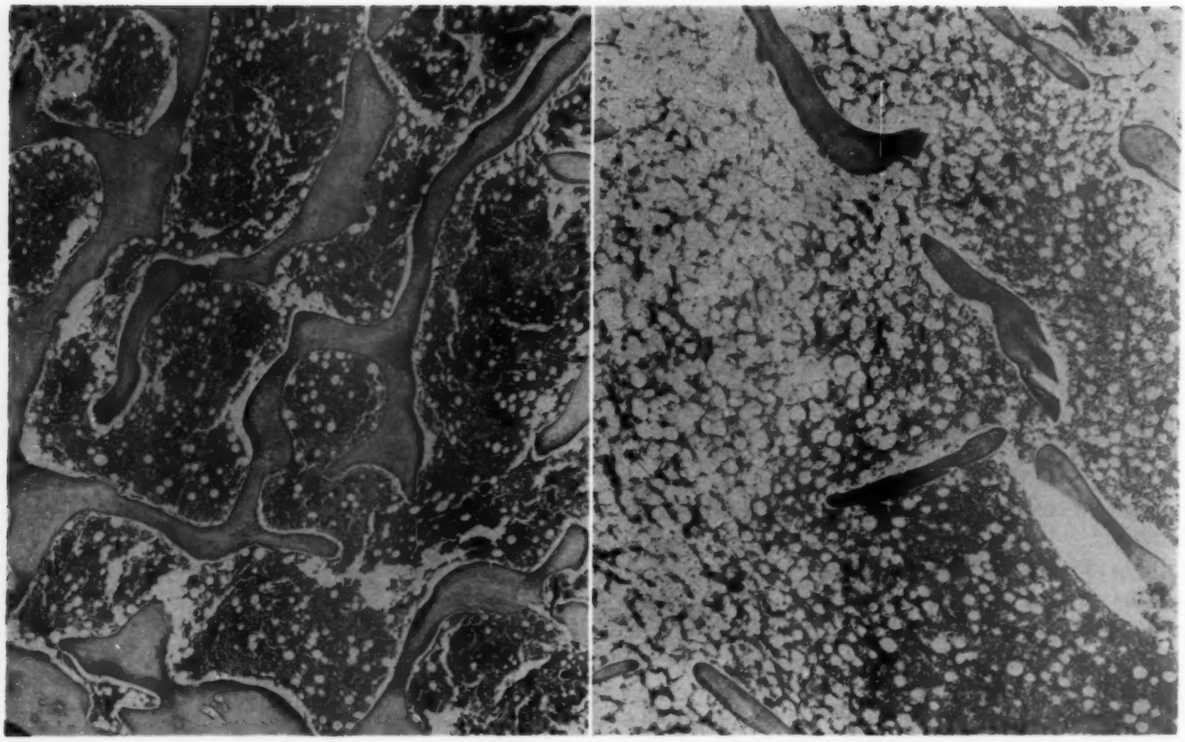


FIG. 1. The spine of a patient with postmenopausal osteoporosis. Note the prominent vertical striations in the vertebral bodies, and the extreme radiolucency of the bone when contrasted with the soft tissues.

The serum alkaline phosphatase, which serves, in the absence of liver disease, as an index of the rate of osteoblastic activity, is normal or low in osteoporosis, as it never is in the other two metabolic bone diseases, characterized by an increased rate of bone matrix formation. (We should expect, if the pathologic physiology of osteoporosis is as outlined here, that the serum alkaline phosphatase would always be low. The fact that "normal" values are found clinically may be explained by the relative insensitivity of the method employed so that "low" values are well within the normal range. In any event, the values found are clear evidence of an inadequate response to the increased strains of the osteoporotic skeleton.)

A number of investigators have described tests designed to establish the diagnosis of osteoporosis by administering calcium "loads," intravenously, and measuring the rate of excretion of calcium in the urine or of its supposed incorporation into bone. The assumptions are made (1) that the rate of incorporation of calcium into bone matrix may be influenced by the level of circulating calcium, (2) that the amount deposited for a given load will be proportional to the bone mass, and (3) that sufficient "lag" will occur



2A 2B

FIG. 2. A, normal vertebral bone from a nineteen year old man. B, osteoporotic bone from a comparable area of a vertebra of an eighty year old woman. Bone in B is histologically normal but the trabecules are thinner and fewer than those in A. Original magnification, × 50.

between the deposition of such infused calcium and the release of comparable amounts from bone that an estimate of bone mass may be made. It is by no means established that the total amount of calcium in normally calcified bone can be increased by increased "pressure" of circulating calcium, although the rate of exchange with surface calcium can very likely be enhanced [*14*]. If the total bone calcium can be increased in this manner, it must be a transient phenomenon, for otherwise an increased supply of calcium would result in increased storage in osteoporosis. It has never been convincingly shown that this is the case, *vide infra*. Indeed, the results of the tests thus far described [*15–17*] have shown that the rate of excretion of infused calcium is not clearly greater in osteoporotic patients than in normal subjects. In one series, however [*17*], the amount estimated to have entered bone in a four-hour period was significantly lower in osteoporosis. In one respect, calcium infusion tests are of established value in the diagnosis of osteoporosis: they serve to exclude osteomalacia, in which the amount of calcium retained is much greater than normal [*15,16*]. Whereas, comparable data are not available for patients with osteitis fibrosa, there appears to be little doubt that they would respond as do patients with osteomalacia. This follows from the well-documented observation [*1*] that changes in calcium intake may produce marked parallel changes in calcium balance in osteitis fibrosa cystica, in contrast to their ineffectiveness in osteoporosis.

In a patient with radiolucent bones, the ultimate criterion for the diagnosis of osteoporosis is an histologic one. Osteoporotic bone resembles normal bone in showing little histologic evidence of bone formation (few osteoblasts, normally narrow or absent osteoid "seams") or of bone destruction (few osteoclasts, absence of fibrosis in the marrow). It differs from normal bone only in that the total mass is less, a point that can be established only when comparable biopsy or autopsy specimens from normal subjects are available. (Fig. 2.) Whereas the disease may thus be distinguished from the other diseases by study of microscopic detail, it is distinguished from

normal bone by low-power examination, from which the overall quantity of bone mass may be evaluated.

The fundamental defect in osteoporosis clearly is a decreased rate of bone matrix formation. In those situations where a direct stimulus to osteoblastic activity has been lost, as is thought to be the case in estrogen deficiency, the histologic observation that very few osteoblasts are present is what might be anticipated. In situations in which the underlying cause of the disorder lies in the unavailability of structural materials, as is thought to be the case in starvation, one might anticipate an increased number of osteoblasts as a compensatory phenomenon. An increase in the number of osteoblasts in the presence of osteoporosis has been found in only two conditions. In fragilitas ossium, this has occasionally been reported [*18*] together with an increase of serum alkaline phosphatase activity. In a rare condition recently described [*19*] as "fibrogenesis imperfecta ossium," osteoporosis appears to be present as a consequence of the deposition of structurally inadequate matrix which cannot calcify to form strong bone despite the presence of normal concentrations of circulating calcium and phosphorus. In this condition there is overproduction of bone matrix, with increased numbers of osteoblasts and an elevation of serum alkaline phosphatase. If the loss of structural materials may indeed be a sufficient cause of osteoporosis, as suggested, the deficiency must in some way affect the osteoblasts as well.

DIFFERENTIAL DIAGNOSIS OF OSTEOPOROSIS

In the differential diagnosis of osteoporosis one must consider on the one hand the metabolic bone diseases, and on the other hand neoplastic disease.

It is generally not difficult to distinguish osteoporosis from osteomalacia and osteitis fibrosa cystica. Radiologically, both of these latter disorders may involve the skull and cause disappearance of the lamina dura. The bilateral, symmetrical, incomplete fractures that characterize osteomalacia, and the bone cysts and subperiosteal bone resorption that characterize osteitis fibrosa are of course not seen in osteoporosis.

The serum phosphorus, which is almost invariably low in osteomalacia and the osteitis fibrosa cystica of primary hyperparathyroidism, and high in the osteitis fibrosa of renal failure, is normal (although sometimes high normal [*10,15*]) in osteoporosis. The serum calcium is of somewhat less diagnostic value. Characteristically normal in osteoporosis, it may be low in osteomalacia and renal osteitis, and is usually high in primary hyperparathyroidism. As has been noted, however, it may be transitorily high in osteoporosis; conversely, it may fall to normal with the onset of renal damage in hyperparathyroidism. The urinary calcium is likewise of limited value: only in osteomalacia of intestinal origin, in which it is very low, does it serve to distinguish osteoporosis from another metabolic bone disease. The serum alkaline phosphatase is of cardinal importance in the differential diagnosis. It is invariably elevated* in osteomalacia and osteitis fibrosa, and is normal in uncomplicated osteoporosis. The commonly observed elevation of the serum alkaline phosphatase in liver disease has provided a major obstacle to the understanding of the bone disease which is commonly present; a definitive diagnosis is virtually impossible without bone biopsy [*21*].

The histologic grounds upon which the differential diagnosis of osteoporosis must ultimately rest have been alluded to earlier.

Invasion of bone by neoplastic disease may result in a clinical picture which resembles osteoporosis, or it may lead to the development of osteoporosis, in the presence of which the primary disease may be overlooked. Under ordinary circumstances, as tumor leads to bone destruction strains on the skeleton are increased locally, and new bone formation is stimulated. The resulting rise in serum alkaline phosphatase may serve to suggest the underlying process. When, however, pain is a prominent feature as it often is with invasion of the spine, it may lead to inactivity, as a result of which the serum phosphatase fails to rise, and osteoporosis supervenes. The differential diagnosis of metastic disease from osteoporosis may be very difficult when no localized lesions can be seen; certainty on this point may ultimately require a biopsy.

One form of malignancy deserves special comment. In multiple myeloma the serum alkaline phosphatase is rarely elevated even in the presence of extensive involvement of bone. Because of this, myeloma may present especial

* The term "chemical-osteomalacia-with-normal-phosphatase" [*20*] is strictly a misnomer; it was introduced to clarify the stages in the development of this disease, and describes a stage before bone disease has developed.

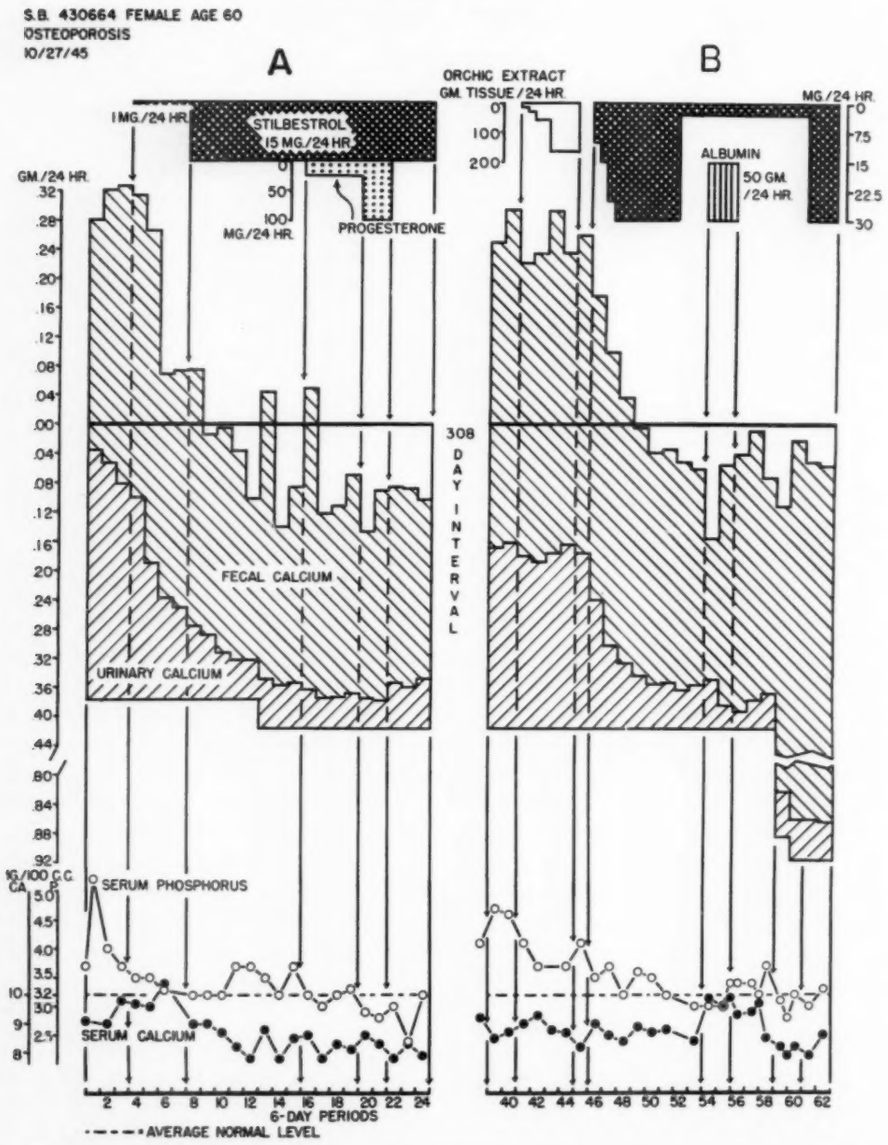


FIG. 3. The effect of stilbestrol and albumin on calcium balance in a sixty-year old woman with postmenopausal osteoporosis and Paget's disease. Note the marked fall in both urinary and fecal calcium, with development of positive calcium balance, in both treatment periods. Balance data are plotted as follows: There is a horizontal base line, and intake is plotted downwards from this line. Urinary and fecal output are plotted upwards from the intake line. If their sum is greater than the intake a hatched area above the zero line indicates negative balance; if it is less, a clear area below the zero line indicates positive balance. Part A is reproduced by permission from: REIFENSTEIN, E. C., JR. and ALBRIGHT, F. The metabolic effects of steroid hormones in osteoporosis. *J. Clin. Invest.*, 26: 24, 1947.

difficulties in differential diagnosis. The apparent failure of compensatory new bone formation in this condition, suggesting a direct effect of the disease on osteoblasts, has not been explained.

TREATMENT OF OSTEOPOROSIS

The treatment of osteoporosis should of course include, when possible, that of the underlying cause. It need not be emphasized that the osteoporosis of starvation or protein lack, Cushing's syndrome, excessive therapy with ACTH or cortisone, uncontrolled diabetes or thyrotoxicosis and scurvy should be approached, whenever possible, with an attack on the basic problem. In addition, four types of treatment of the bone disease itself have been found effective: mobilization, estrogens, androgens and albumin.

Administration of calcium, with or without

vitamin D, is of little or no benefit in osteoporosis. It does not produce sustained positive calcium balance, and does little to relieve symptoms. This is of course what one would anticipate in a disorder wherein the organic matrix, rather than the mineral components of bone, is at fault. There is, moreover, a danger in such therapy; in some subjects it may, by increasing the urinary calcium, increase the danger of forming renal calculi. In Figure 3, periods 59 through 62, a study is shown in which a two-fold increase in the calcium intake was without effect on the calcium balance. It is also apparent from the figure that in this subject the increase in calcium intake (as calcium gluconate) produced no change in the absorption of calcium, appearing merely as an increase in fecal calcium.

In all forms of osteoporosis it is important that the patient be kept as mobile as possible. If this is not done, as it frequently is not because of severe bone pain, further osteoporosis of disuse will be superimposed on the original disorder. This will in turn lead to more bone pain and it may be difficult or impossible to terminate the "feed back" so established. Osteoporosis in a real sense "taketh from him who hath not even that which he hath."

Estrogens are effective, and indeed specific in the treatment of postmenopausal osteoporosis. They are regularly effective in senile osteoporosis and in the osteoporosis of disuse and of Cushing's syndrome [*1*]; little or not at all effective in osteogenesis imperfecta [*22*]; and completely without effect in idiopathic osteoporosis. As already noted, a therapeutic test of estrogens under balance regimen may be of value in ruling out, in a given case, any known cause of osteoporosis. In Figure 3 is shown the response of the calcium balance to estrogen therapy on two occasions in a patient with postmenopausal osteoporosis complicating Paget's disease of bone. The patient was in markedly negative calcium balance before therapy was begun, and the urinary calcium was very high (350 and 250 mg. a day, respectively, in the two studies). With stilbestrol treatment a positive calcium balance was established, and the urinary calcium fell to 40 and 60 mg. a day. Estrogen therapy is often dramatically effective in relieving the pain of osteoporosis. (The mechanism of this effect, which is often too rapid to represent new bone formation, is not understood.) It can regularly be shown by appropriate studies to induce positive calcium balance. It should be added that it is

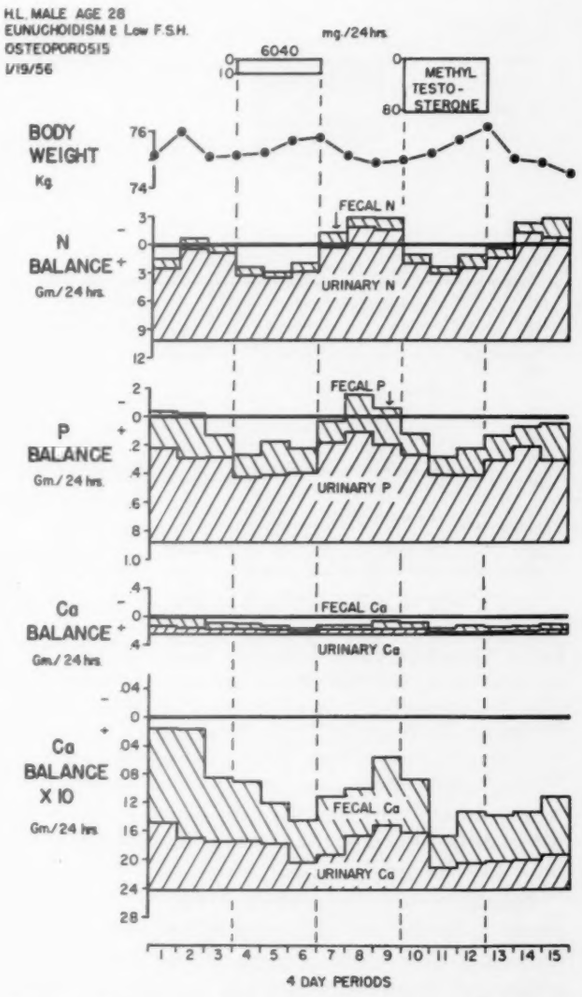


FIG. 4. The effect of androgens on a twenty-eight year old man with eunuchoidism and osteoporosis. Note that both types of androgen induced markedly positive nitrogen balance and moderate retention of calcium. "6040" is 9-fluoro-11-hydroxyl-17 methyl testosterone, (Halotestin*). For method of plotting, see legend to Figure 3.

rarely possible to produce unequivocal radiologic improvement with estrogens or any other medication in osteoporosis with the sole exception of that of Cushing's syndrome, which may show unequivocal increase of density with successful therapy. This apparent discrepancy between the results readily obtained during short balance studies and those of long-term treatment suggest that there is some limit to the amount of bone formation which can be induced with estrogens in man. It may be, for example, that whereas estrogens can induce deposition of bone in apposition to existing bone,

* Provided by The Upjohn Co., Kalamazoo, Mich.

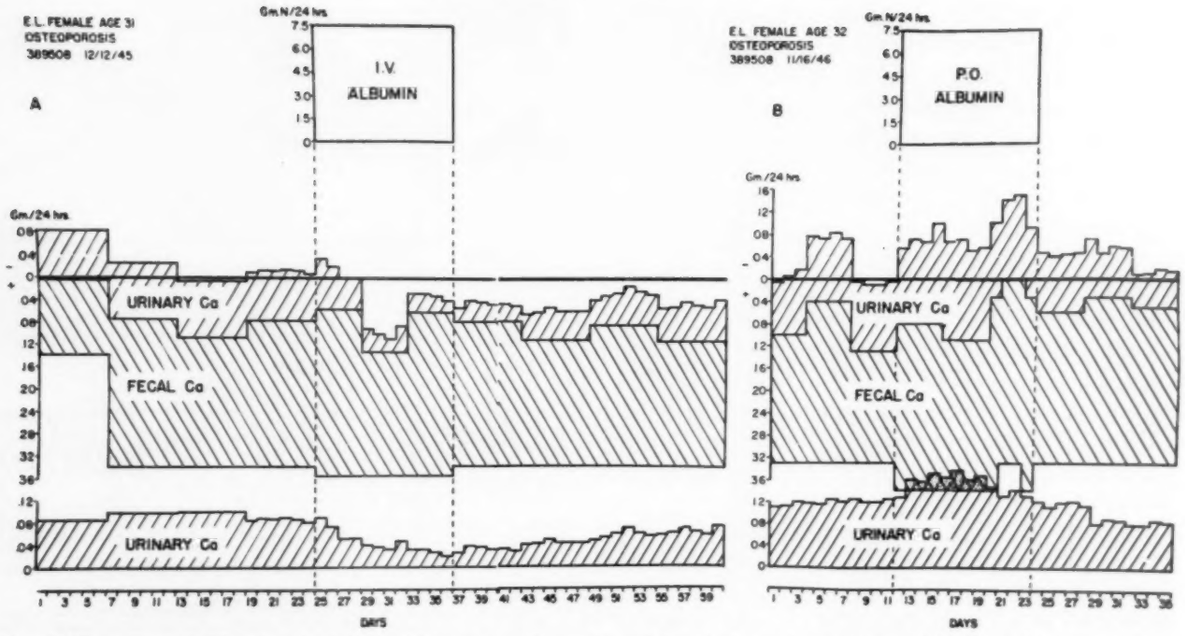


FIG. 5. The effect of albumin administered intravenously on the calcium balance in a patient with idiopathic osteoporosis (A) contrasted with that of the same amount of albumin administered orally (B). Note prolonged calcium retention in (A). The amount of calcium retained strongly suggests that formation of new bone has occurred. Reproduced from: ALBRIGHT, F., BARTTER, F. C., DEMPSEY, E. F., FORBES, A. P., HENNEMAN, P. H. and REIFENSTEIN, E. C., JR. Serum albumin and bone matrix. Transactions Fifth Conference on Metabolic Interrelations, p. 277. New York, 1953. Josiah Macy, Jr. Foundation.

they cannot initiate the restoration of trabeculae once lost in osteoporosis.

Estrogen will often reverse the negative calcium balance of the untreated osteoporotic patient in doses approaching physiologic ones, as estimated from their effects upon the endometrium. Thus stilbestrol in doses of 1 mg. a day may be as effective as much larger doses (for example, 30 mg. a day). See Figure 3.

Androgen therapy is effective in those forms of osteoporosis in which androgen lack is in part responsible for the underlying disorder. These include the osteoporosis of male eunuchoidism and senile osteoporosis. Calcium retention induced by androgens is always (at least under experimental conditions) associated with nitrogen retention, and the results suggest that the effect on bone matrix is part of the general nitrogen anabolic effect of the steroids. In Figure 4 is shown the response of the calcium balance to androgen therapy in a male patient with eunuchoidism and osteoporosis. He was completely immobilized both before and during the study as a result of slipped femoral epiphyses, so that changes in activity play no part in the results.

The effect of small doses of androgen, for example, 10 mg. of methyl testosterone a day, on calcium balance has not been adequately studied. The doses which have been shown to be effective in the repair of osteoporosis (25 mg. of testosterone propionate, or 40 to 100 mg. of methyl testosterone a day) [*23*] cannot be tolerated by female patients for long periods without danger of masculinization.

There is clinical evidence to suggest that, even at a dosage of 5 to 10 mg. a day by linguet, methyl testosterone is a valuable adjunct to the treatment of osteoporosis with estrogens. Androgen therapy may be of value also in preventing the osteoporosis which frequently complicates treatment with ACTH and cortisone and related steroids. It can readily be shown to prevent the nitrogen loss induced by these steroids [*24,25*], and it is likely that the effects on calcium balance are secondary to this.

Human serum albumin is the only agent which has been shown to induce calcium retention in all forms of osteoporosis [*26,27*]. The calcium retained can be shown to be greatly in excess of that bound to the administered albumin, and to persist in bone when most of the albumin has been degraded. The same amount of albumin administered orally produces no

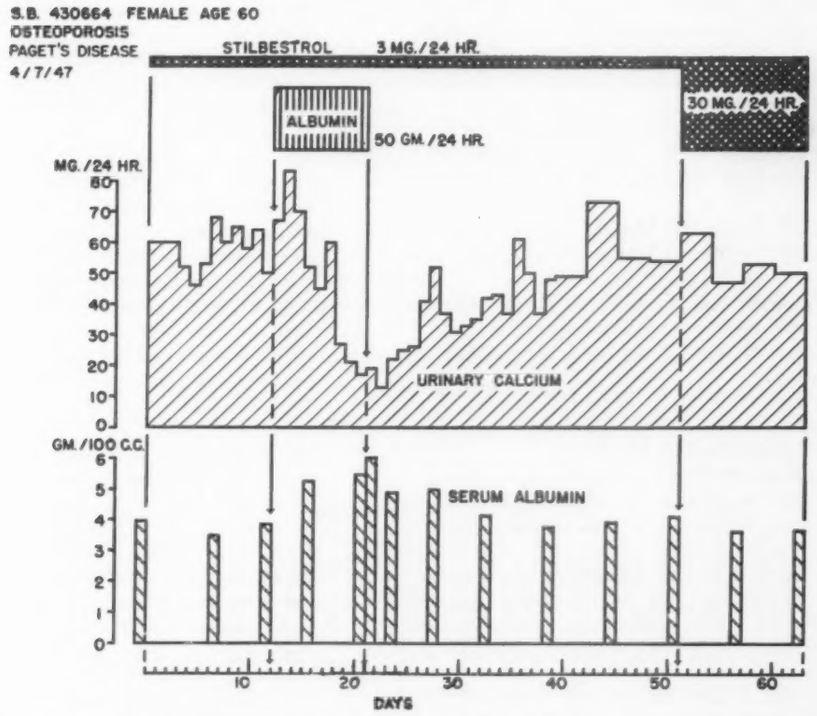


FIG. 6. Detail from the study shown in Figure 3B. The effect of albumin administered intravenously on the urinary calcium during the study shown in Figure 3. Note that albumin-induced calcium retention is well in excess of that occurring as a result of estrogen therapy. Reproduced from ALBRIGHT, F., BARTTER, F. C., DEMPSEY, E. F., FORBES, A. P., HENNEMAN, P. H. and REIFENSTEIN, E. C. JR., Serum albumin and bone matrix. Transactions Fifth Conference on Metabolic Interrelations, p. 277. New York, 1953. Josiah Macy, Jr. Foundation.

calcium retention. For these reasons it has been suggested that serum albumin or some incompletely hydrolyzed component thereof may be a precursor of bone matrix. In Figure 5 is shown the response of the calcium balance to intravenous albumin administration in a patient with idiopathic osteoporosis, contrasted with the response to oral albumin. The response in a patient with postmenopausal osteoporosis and Paget's disease is shown in Figure 6: the effect is here superimposed on that of estrogen shown in Figure 3. In contrast to this latter patient, the former had presented as a young woman with no suggestion of endocrine disease. Large doses of estrogens had been without effect on her calcium balance.

SUMMARY

Osteoporosis is that metabolic bone disease in which the bone mass is reduced as a result of an inappropriately low rate of bone matrix formation. It is the only one in which circulating calcium, phosphorus and phosphatase concentrations are normal. It may result from inactivity, from estrogen or androgen lack, from excess of adrenal cortical hormones, from a deficiency of nitrogenous precursors, or in the absence of known cause.

Mobilization, and the indicated specific measures, are of importance in therapy. Of great academic interest and of possible importance in therapy is the response of all forms to intravenously administered human serum albumin.

REFERENCES

1. ALBRIGHT, F. and REIFENSTEIN, E. C., JR. Parathyroid Glands and Metabolic Bone Disease. Baltimore, 1948. Williams & Wilkins Co.
2. KOCH, J. C. The laws of bone architecture. *Am. J. Anat.*, 21: 177, 1917.
3. GARDNER, W. V. and PFEIFFER, C. A. Influence of estrogens and androgens on the skeletal system. *Physiol. Rev.*, 23: 139, 1943.
4. MCLEAN, F. C. and URIST, M. R. Bone; an Introduction to the Physiology of the Skeletal Tissue. Chicago, 1955. University of Chicago Press.
5. ARMSTRONG, W. D., KNOWLTON, M. and GOUZE, M. Influence of estradiol and testosterone propionates from skeletal atrophy from disuse and on normal bones of mature rats. *J. Endocrinol.*, 36: 313, 1945.
6. SISSONS, H. A. The osteoporosis of Cushing's syndrome. *J. Bone & Joint Surg.*, 38B: 418, 1956.

7. Follis, R. H., Jr. Diseases, Particularly of Bone, Associated with Derangement of Calcium and Phosphorus Metabolism, Metabolic Interrelations. Fifth Conference, p. 196. New York, 1953. Josiah Macy Jr. Foundation.
8. Krane, S. M., Brownell, G. L., Stanbury, J. B. and Corrigan, H. The effect of thyroid disease on calcium metabolism in man. *J. Clin. Invest.*, 35: 874, 1956.
9. Key, J. A. Brittle bones and blue sclera. Hereditary hypoplasia of the mesenchyme. *Arch. Surg.*, 13: 523, 1926.
10. Albright, F., Bartter, F. C. and Reifenstein, E. C., Jr. The Fourteenth Conference on Convalescence, p. 102, New York, 1946. Josiah Macy, Jr. Foundation.
11. Mack, P. B., Brown, W. N., Jr. and Trapp, A. D. The quantitative evaluation of bone density. *Am. J. Roentgenol.*, 61: 808, 1949.
12. Albright, F., Burnett, C. F., Cope, O. and Parson, W. Acute atrophy of bone (osteoporosis) simulating hyperparathyroidism. *J. Clin. Endocrinol.*, 1: 711, 1941.
13. Reifenstein, E. C., Jr. and Albright, F. Paget's disease: its pathologic physiology and the importance of this in the complications arising from fractures and immobilization. *New England J. Med.*, 231: 343, 1944.
14. Dallemagne, M. J. and Fabry, C. Structure of Bone Salts. Bone Structure and Metabolism, Ciba Foundation Symposium. London, 1956. J. A. Churchill, Ltd.
15. Lichtwitz, A., de Sèze, D., Hioco, D. and Bordier, P. L'hypercalciurie provoquée, test dynamique d'exploration du squelette. *Semaine d. hôp. Paris*, 31: 3789, 1955.
16. Nordin, B. E. C. and Fraser, R. A calcium-infusion test. I. Urinary excretion data for recognition of osteomalacia. *Lancet*, 1: 823, 1956.
17. Finlay, J. M., Nordin, B. E. C. and Fraser, R. A calcium-infusion test. II. Four-hour skeletal retention data for recognition of osteoporosis. *Lancet*, 1: 826, 1956.
18. Smith, O. N. and Mitchell, J. McK. Serum phosphatase in osteogenesis imperfecta. *Am. J. M. Sc.*, 190: 765, 1935.
19. Baker, S. L. Fibrogenesis imperfecta ossium: a generalized disease of bone characterized by defective formation of the collagen fibres of the bone matrix. *J. Bone & Joint Surg.*, 38-B: 378, 1956.
20. Albright, F., Burnett, C. H., Parson, W., Reifenstein, E. C., Jr. and Roos, A. Osteomalacia and late rickets. *Medicine*, 25: 399, 1946.
21. Schmid, R., Johnson, L. and Bartter, F. C. Studies in the diagnosis and treatment of osteoporosis in a patient with biliary cirrhosis. In preparation.
22. Ropes, M. W., Rossmeisl, E. C. and Bauer, W. The Fourteenth Conference on Metabolic Aspects of Convalescence. Treatment of Osteogenesis Imperfecta. p. 87. New York, 1946. Josiah Macy, Jr. Foundation.
23. Reifenstein, E. C., Jr. and Albright, F. The metabolic effects of steroid hormones in osteoporosis. *J. Clin. Invest.*, 26: 24, 1947.
24. Bartter, F. C., Fourman, P., Albright, F., Jefferies, W. McK., Dempsey, E. and Carroll, E. L. Does methyl testosterone modify the effects of adrenocorticotropic hormone (ACTH) and of desoxycorticosterone glucoside (DOCG)? Symposium on Pituitary-Adrenal Function. 1949. American Association for the Advancement of Science.
25. Reifenstein, E. C. Jr. The rationale for the use of anabolic steroids in controlling the adverse effects of corticoid hormones upon protein and osseous tissues. *South. M. J.*, 49: 933, 1956.
26. Albright, F., Forbes, A. P., Bartter, F. C., Reifenstein, E. C., Jr., Bryant, D., Cox, L. D. and Dempsey, E. F. Studies on the fate of intravenously administered human plasma proteins in idiopathic hypoproteinemia and in osteoporosis. Symposia on Nutrition, Plasma Proteins., vol. 2. Robert Gould Research Foundation, Inc., 1950.
27. Albright, F., Bartter, F. C., Dempsey, E. F., Forbes, A. P., Henneman, P. H. and Reifenstein, E. C., Jr. Serum albumin and bone matrix. Transactions Fifth Conference on Metabolic Interrelations, p. 277. New York, 1953. Josiah Macy, Jr. Foundation.

Combined Staff Clinic

Metabolic and Clinical Aspects of Gout

THESE are stenotyped reports of a Combined Staff Clinical Conference of the National Institutes of Health, presented by the staff of the National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland. The reports are edited by Dr. J. E. Seegmiller.

DR. DEWITT STETTEN, JR.: The subject of gout is one which has for many years been a borderline subject between the chemist and the physician. The chemical history can be mentioned in just a few sentences.

Uric acid was apparently first discovered by Scheele, the discoverer of oxygen, prior to 1800 in a urinary calculus. Urinary calculi appeared to have fascinated the chemists of that time because Wollaston a few years later, while studying a urinary calculus, first discovered cystine. Wollaston is connected with the story of gout because he first demonstrated the presence of sodium urate in a tophus which purportedly he dug out of his own ear. It was a quarter of a century later that Garrod [*1*] performed his historic experiment in which he took serum from a patient with gout, inoculated it with a vegetable fiber, and placed it on his mantelpiece overnight. The following morning he noted that this thread was encrusted in crystals which he identified as sodium urate. He proposed this as a diagnostic test for the disease and, with minor modifications and the addition of a Beckman spectrophotometer, this is still an aid to diagnosis. I mention these things because I think they show the chemist, as well as the clinician, has a vested interest in this disease.

We are starting our program this afternoon with a case presentation by Dr. Laster.

DR. LEONARD LASTER: The patient is a twenty-two year-old white male student from North Carolina who was referred from Johns Hopkins Hospital for studies of purine metabolism.

At the age of five, he suffered recurrent pains in both heels, so severe that he was seen by a physician who, however, detected no diagnostic abnormalities. In time these pains subsided. At the age of twelve the patient again noted pain in both feet and in his lower back. These were persistent complaints for about half a year and then subsided uneventfully.

At the age of fifteen, during a strenuous football season, the patient noted recurrent pains in the distal ends of the metatarsal bones of his feet. These pains subsided in a few months and he was asymptomatic until the next football season when he played in his first varsity game. The following morning he awoke to find his right big toe red, hot, swollen and exquisitely tender. An x-ray film revealed a cystic lesion in the bone involved by the acute process. Surgery was advised and during the operation the diagnosis of gout was suspected for the first time. Thereafter, clinical studies including determination of serum uric acid established the diagnosis.

The patient was treated successfully with colchicine and given a low purine diet for a brief period of time. He recovered uneventfully from his acute attack. Since then the patient has had about ten to twenty attacks of arthritis, only two of which have been severe enough to confine him to bed. These attacks all responded to colchicine taken in quantities sufficient to cause diarrhea. The seizures have involved the hands, shoulders, feet, ankles and on rare occasions the knees.

At the age of seventeen he noted, for the first time, the appearance of "little nodules" in the pulps of his fingertips. Since then tophaceous deposits have involved the fingers, big toes, elbows, ears, knees and tendons, such as the flexor tendons of the hands and the Achilles tendon of one foot. Initially the deposits increased in size very rapidly and caused marked damage to the bones in the hands and feet. About one and a half years ago, following an injury sustained while participating in sports, a hematoma developed in the tophus on the patient's right elbow. This subsided without requiring surgery. More recently a tophus on one foot became infected following a minor abrasion and an area of necrosis developed that was slow to heal.

At the age of twenty, following a severe attack of acute gout which responded to colchicine, the patient went to the Johns Hopkins Hospital where he was studied. The results of laboratory tests were: Serum uric acid, 10.2 mg. per cent; non-protein nitrogen, 58 mg. per cent; phenolsulfonphthalein excretion, 48 per cent in two hours; urine concentration test, maximum specific gravity, 1.013; urinary sediment, negative. An intravenous pyelogram was attempted but because of very poor concentration of the dye the kidneys were not adequately visualized. His blood pressure was

1A

1B

1C

FIG. 1. Chronic tophaceous gout in a twenty-one year old man. A, elbows; B, feet; C, hands.

160/110 and during subsequent visits it was recorded at similarly high levels.

The patient was started on a regimen of 2 gm. of benemid® and 4.5 gm. of sodium bicarbonate a day. A fortnight later an acute attack of gout developed which responded well to colchicine and thereafter his therapeutic regimen was supplemented with 0.6 mg. of colchicine a day. He remained on this program and returned to Johns Hopkins Hospital one year later. At that time both he and his physician thought the tophaceous deposits had not changed in size. Although they had not decreased, the previous relentless progression appeared to have ceased. His serum uric acid was 14 mg. per cent; non-protein nitrogen 76, mg per cent; phenolsulfonphthalein excretion, 26 per cent in two hours. In an attempt to lower the serum uric acid the dose of benemid was increased to 3 gm. a day. Since then the patient has suffered occasional minor attacks of gout and no marked changes have occurred in his tophi.

His past history reveals no symptoms suggestive of renal calculi. He had enuresis until the age of fifteen; examination of the urine at that time was reported as negative.

The family history is significant. When the patient was referred to the Clinical Center in May, 1955, his mother accompanied him and during this visit she gave a history of having suffered marked pain in her right big toe during the preceding five weeks. Her serum uric acid level was found to be 10 mg. per cent and she was admitted to the Clinical Center with her son for evaluation of her gout. While under observation typical acute gout developed which responded to colchicine. In addition, the patient's maternal uncle, now aged forty-one, suffered an attack of acute arthritis at the age of twenty-six, while playing basketball, and since that time has had recurrent acute episodes of arthritis. After his nephew was found to have gout, the same diagnosis was established in this uncle and he too has been studied at the Clinical Center, where his serum uric acid was found to be 11 mg. per cent.

The rest of the system review and past history are non-contributory.

On physical examination of the patient, a tall, thin, muscular youth, the blood pressure was found to be 130/70, pulse rate 58 beats per minute. There were no significant changes in the retinas. The rest of the positive findings were limited to the joints and sites of tophaceous deposits. The pinna of the left ear bore several small tophi and that of the right ear had a soft, fluctuant, non-tender swelling in addition to a tophaceous deposit. The extremities showed marked involvement of the interphalangeal joints and flexor tendons of several fingers, with massive tophaceous deposits. The wrists were similarly involved with urate deposits that caused limitation of motion. The tophaceous deposits about the elbows, feet and hands are shown in Figure 1. The one on the right elbow was

stony hard and immovable, that on the left elbow was a soft, fluctuant mass with loose, gritty material inside it. The spine showed no clinical evidence of involvement. The knees had several minor tophaceous deposits. The Achilles tendon of the right foot carried three large tophi. The toes were severely involved, with massive tophaceous deposits and marked impairment of function. No joints were tender, warm or red. Laboratory tests revealed: Hematocrit, 39 per cent; white blood count, 5,400 per cu. mm. with 51 per cent polymorphonucleocytes, 37 per cent lymphocytes, 6 per cent monocytes and 6 per cent eosinophils. The eosinophilia was noted on several occasions and no cause for it could be determined. Urinalysis: No albuminuria; specific gravity, 1.009; urinary sediment, rare red blood cell, occasional white blood cell. The serum uric acid was 10.5 mg. per cent. X-ray studies showed moderately advanced gouty changes involving multiple joints of the feet, ankles, elbows, wrists, hands and spine. Involvement of the spine was considered a most unusual finding in gouty arthritis. Retrograde pyelography revealed an abnormal appearance of the renal calyces that suggested deposits in the collecting systems bilaterally. In addition, there was suggestive evidence for the presence of a stone in the left renal pelvis.

In order to study the excretion of uric acid the patient was given a purine-free diet that was constant in protein and caloric intake. When he was taking 3 gm. of benemid a day the urinary excretion of uric acid was 341 mg. per day and the serum level was 10.5 mg. per cent. Following omission of benemid the urinary excretion dropped to 200 mg. per day with no change in the level of serum uric acid. Initially after starting sodium salicylate, 4.5 gm. per day, the urinary excretion of uric acid was 550 mg. per day and then leveled off at 450 mg. per day, with an associated drop in the serum uric acid to 8.9 mg. per cent. He was discharged therefore on a regimen of 4.5 gm. of sodium salicylate a day as a uricosuric agent and 4.5 gm. of sodium bicarbonate a day as an alkalizing agent to prevent urate deposition in the genitourinary tract. Symptoms of salicylism prevented the use of higher doses of salicylate.

He was seen again in August, 1955, when he reported no obvious changes in his tophi. He thought one tophus on the left pinna was somewhat smaller and reported that another one had drained spontaneously. In addition, he had recently abraded his left big toe while playing tennis and it had not healed. General physical examination and laboratory studies were as before. His blood urea nitrogen was 58 mg. per cent and the fasting serum uric acid was 9.5 mg. per cent. On a purine-free diet and continued salicylate therapy the urinary uric acid excretion was 450 mg. per day. During this admission the infected toe was treated with conservative measures and improved slowly. In addition, the large tophi were removed from both elbows and the surgical specimens were

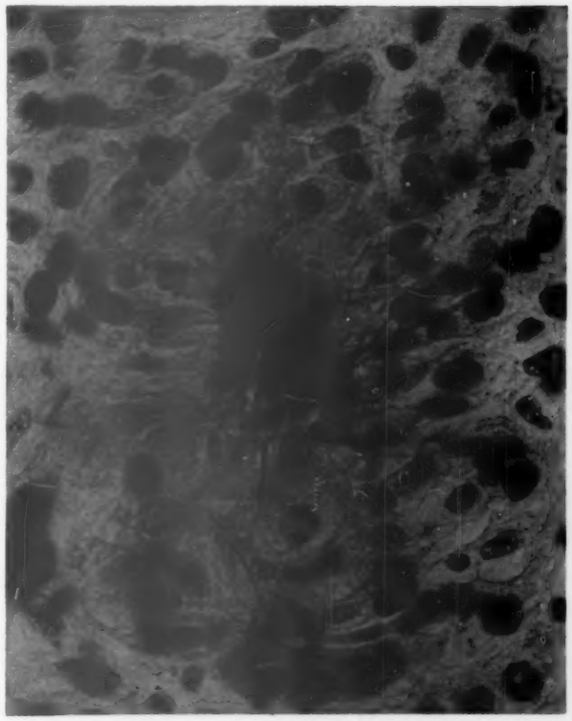


FIG. 2. Early urate deposits in synovial membrane during acute gout.

studied pathologically and chemically. Since this procedure the tophectomy site on the left elbow has healed, the one on the right drained serous fluid following the procedure and about the middle of September his school physician removed some necrotic tissue and opened it to drain. Since that time, the discharge has diminished somewhat, but still persists. He has had no acute attacks of gout.

In summary, the following points are of interest in this patient: (1) The family history of gout. (2) The onset at an early age and the possible initial relationship to trauma. (3) The rapid progress of the tophaceous aspect of this malady. (4) The complications of this disease, such as destruction of joints by the tophi, secondary infection of the tophaceous deposits and the marked impairment of renal function. (5) The difficulty in managing this patient is that he does not achieve a satisfactory uricosuria from benemid, presumably due to his renal impairment.

QUESTION: Has he ever had any fine gravel or fine stones in his urine in the past?

DR. LASTER: The patient gives no history of gravel in the urine or renal pain.

QUESTION: Are there any precipitating causes for the attacks?

DR. LASTER: After detailed questioning we could find no obvious precipitating causes. He has, through the years, continued to play golf

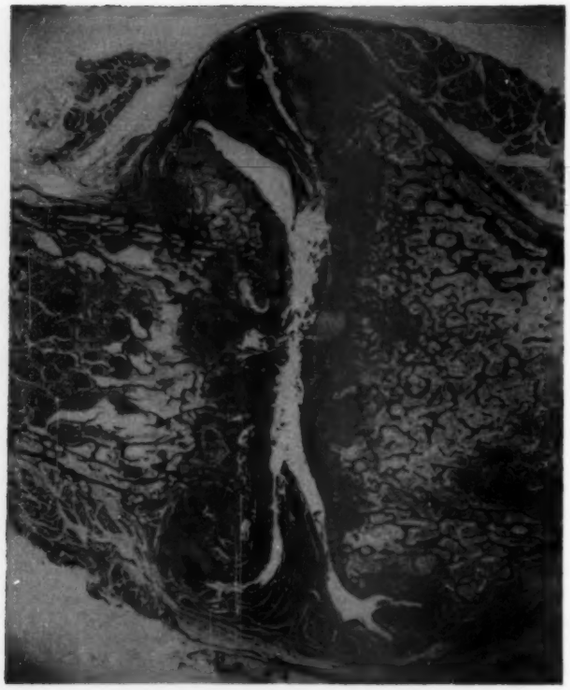


FIG. 3. Advanced urate deposits in articular cartilage, subchondral bone and synovial membrane of patient with gout. (From: Sokaloff. *Am. J. Clin. Path.*, 24: 406, 1954.)

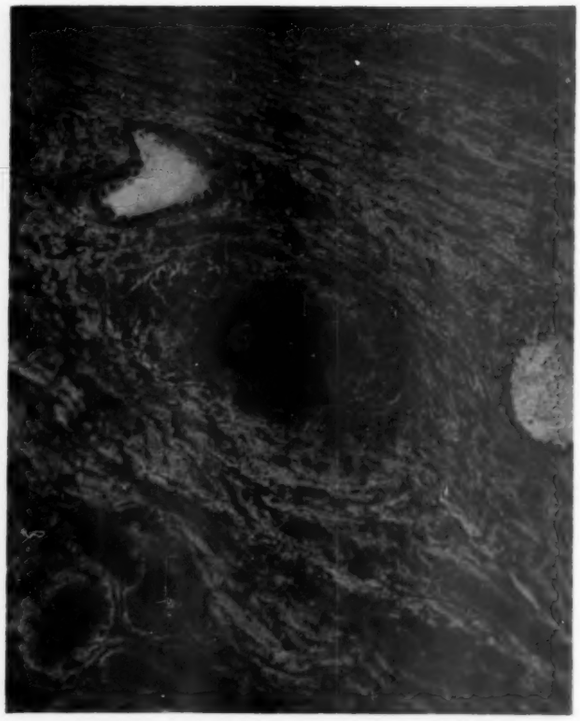


FIG. 4. Lipid matrix materials associated with urate deposits as revealed by oil red O stain.

and tennis, and this exertion and trauma do not seem to have brought on acute attacks. He cannot relate acute attacks to the ingestion of any specific foods or drinks. He does not consume much alcohol in any form. During the period we have studied him, we have noted no precipitating factors to his attacks.

DR. STETTEN: Thank you, Dr. Laster. As you will have noted, the tophi were removed and studied here. Dr. Sokoloff will report to us on the pathologic findings of these specimens.

DR. LEON SOKOLOFF: The characteristic lesion of gout is the tophus or foreign body granuloma which forms about the deposits of urate crystals.

A section of synovial membrane from a patient who was experiencing an acute attack of gouty arthritis is shown in Figure 2. The section has been prepared by de Galantha's method which is a simple and valuable method for demonstrating water-soluble biurate crystals that are not ordinarily preserved in routine histologic preparations. The presence of individual urate crystals, singly and in clumps, would seem to represent an early stage in the formation of a tophus. At times this is accompanied by an acute purulent inflammatory component. Figure 3 illustrates advanced changes. Urate crystals appear as a dark black material. In this photograph they are deposited in the articular cartilages, in the chondral bone and synovial membrane. As a result of these deposits the articular cartilage is becoming eroded in many areas and there is a complicating development of osteoarthritis with large marginal osteophytes.

The tophus that was excised from the elbow region of the patient presented here today involved the wall of the bursa of the elbow, but not the bursal space. It had a characteristic appearance microscopically. By means of a special stain it was possible to demonstrate things that are not commonly appreciated. In Figure 4 we see a frozen section that was stained with oil red O. Large amounts of lipid material were found in the areas of crystal deposition and constituted a large portion of the matrix substance in which the crystals lay. In other areas of the tophus, lipid material was not present in the matrix. Stained with toluidine blue, metachromatic intercrystalline material appeared violet in contrast to the blue-stained surrounding connective tissues.

These sections illustrate the fact that at some stages in the evolution of the tophus, complex

matrix materials are associated with the crystals. At the present moment it is not possible to state that these materials represent a response of the tissues to the presence of urate crystals. It is an intriguing thought that they may be responsible in some manner for the deposition of the crystals. Undoubtedly they constitute an important part of the tophus.

The renal lesions of gout present another area of uncertainty in the pathogenesis of the disease. Although a urate crystal nephrosis is undoubtedly present in a large number of patients with gout, it is most often of limited proportions. Arteriolar nephrosclerosis and chronic pyelonephritis are common pathologic findings in patients with gout. The relation these bear to the gout crystal nephrosis is not clear. In the present instance the possibility that the patient has a urate calculus may offer an opportunity to obtain biopsy tissues that will elucidate the nature of his renal lesion.

DR. STETTEN: Thank you, Dr. Sokoloff. Dr. Bunim will now review the clinical management of this disease.

DR. JOSEPH J. BUNIM: The case just presented is unusual but not unique. Gouty arthritis, and especially tophaceous gout, rarely occurs in teenagers. Yet Garrod reported instances of acute gout before the age of ten. This patient experienced his first attack at age sixteen. Very likely he had hyperuricemia for years before his initial bout of arthritis.

The family history is typical. In this case the trait was inherited from the maternal side. The mother of this patient and her brother were studied at this Institute; they both have hyperuricemia and gouty arthritis. The mother's father and two sisters had renal calculi but we do not know whether or not they had hyperuricemia. A history of gouty arthritis in these three members was denied. The passage of renal calculi, or indeed the appearance of tophi, may precede the first attack of acute gouty arthritis. About 25 per cent of the relatives of patients with gout have hyperuricemia, the vast majority being males [*2*]. Of those members who have elevated uric acid in the blood, only about 10 per cent of the heterozygotes for this factor present clinical manifestations of gout [*3*]. Hyperuricemia in family members is due to a single, autosomal, dominant gene. Although 95 per cent of patients with overt gout are males, the trait of hyperuricemia is not sex-linked. It is believed that heterozygous women are less likely to have elevated serum urate because of a lower normal blood level in females, and of a lessened effect of the abnormal gene in women.

The age of onset of gouty arthritis in this patient was sixteen, in his maternal uncle, twenty-three, and in his mother it was fifty, following her menopause. Hippocrates stated that he did not observe a case of gout in females before the menopause. It is also typical that in the two young male members the disease is severe and tophaceous; in the middle-aged woman it is relatively mild and non-tophaceous.

Not uncommonly, an attack of gout is precipitated by a surgical procedure and the diagnosis should be considered whenever acute arthritis occurs postoperatively. In this patient, however, the acute articular episode appeared first and the diagnosis of podagra was made by the surgeon after he cut into the first metatarsal bone ostensibly to enucleate a bone cyst. Following the operation the blood was analyzed and hyperuricemia discovered.

During the first year of this patient's illness several acute brief bouts of arthritis occurred and he was, characteristically, completely symptom-free between attacks. At the end of only one year tophi appeared. It was apparent then that the patient had embarked quite early on a severe course of chronic tophaceous gout. One is tempted to label this a case of "juvenile gout," reminiscent of juvenile diabetes in severity and in difficulty of management.

Four years after the onset of arthritis, at age twenty, renal disease had developed with progressively increasing azotemia, decreasing phenolsulfonphthalein excretion and fixed specific gravity of the urine. It is noteworthy that persistent hypertension, albuminuria and cylindruria are absent. Modern and Meister [*4*] recently drew attention to this distinctive combination of abnormal renal findings and thought it was characteristic of the tophaceous "kidney of gout." They believed the intrinsic renal lesion in gout is an ascending one, due to compression of the collecting tubule by the pyramidal and medullary urate deposits and eventual atrophy of the nephron which the tubule subserves. There is no unanimity, however, among many qualified observers as to the pathogenesis of renal disease in gout. Chemical injury resulting from filtration of excessive amounts of relatively insoluble urates and ischemia following vascular sclerosis in conjunction with secondary infection and pyelonephritis

seem to constitute a reasonable explanation of the diverse renal lesions found at autopsy.

The treatment this patient received conforms well with what is currently recommended, although it has not led to spectacular results in this particular case. Although colchicine has been used to abort acute attacks of gout for more than 1,400 years, satisfactory measures for prophylaxis and treatment of chronic tophaceous gouty arthritis have evolved only in the past five years [5].

The management of gouty arthritis varies with the type of the attack and the stage and severity of the disease. Acute attacks not only can be aborted quite satisfactorily but can also be prevented.

In acute gouty arthritis colchicine given orally in doses of 0.6 mg. every hour until nausea or diarrhea develops is almost always effective; so much so, that this drug is used as a diagnostic test for gout. A total of 8 mg. is the average amount required. Colchicine administered intravenously may be more satisfactory, since it acts sooner and in most instances terminates the attack before inducing objectionable gastrointestinal symptoms. We have found the intravenous route effective in patients who did not respond to oral administration of colchicine. Doses of 1 or 2, or sometimes 3 mg. in 20 cc. of saline are given as early in the attack as possible and repeated if necessary four to eight hours later. Following the acute attack oral colchicine, in doses of 0.6 mg. two or three times daily, is continued for about a week. If the patient has more than one or two attacks a year it is worthwhile instituting prophylactic therapy which consists of 0.6 to 1.8 mg. each night before retiring.

Phenylbutazone has also been found to be quite effective and usually terminates an acute episode in twenty-four to forty-eight hours. In the first dose 400 mg. are given orally and 100 mg. every four hours thereafter for four more doses. On the second day 100 mg. are given every six hours for four doses following which daily maintenance doses of colchicine are given, as previously mentioned. When given for only two days phenylbutazone is rarely, if ever, toxic and, unlike colchicine, its administration is unaccompanied by unpleasant intestinal symptoms.

Corticotropin given intramuscularly in a single injection of 100 mg. is also a very useful therapeutic agent in acute gouty arthritis.

The pathogenesis of acute gouty arthritis is not understood. It is probably neither related to hyperuricemia nor is it caused by urate deposits in the joint structures. The treatment of an acute attack therefore remains empirical. It is one of the engimas of medicine that colchicine, which does not affect uric acid metabolism, aborts and often prevents acute attacks of gouty arthritis, whereas probenecid (benemid), which is a very potent uricosuric agent, is of no value during the acute episode.

The pathogenesis of chronic gouty arthritis is more clearly understood. It is due to urate deposits in the tissues of and near the joints. Uricosuric agents such as probenecid (benemid) or salicylates in adequate doses inhibit the reabsorption of urates at the proximal tubule and thus increase urinary excretion, usually restore to normal the concentration of uric acid in the blood and interstitial fluid, and consequently mobilize the urates in the tissues. Provided probenecid is taken faithfully and without interruption for prolonged periods (several years), new tophi do not appear and pre-existing tophi do not increase in size. Not infrequently, the tophi soften and shrink in size and occasionally disappear. The required daily dose of probenecid will vary from 0.5 to 2 or 3 gm., according to the uricosuric response of the individual patient, the degree of excess of urates in the body, the patient's daily intake of purines and fats, the severity of functional impairment of the kidneys and the susceptibility to renal calculi. After maximum mobilization of urates has been achieved, the maintenance or prophylactic dose may be lowered. In about 10 per cent of the cases probenecid must be discontinued because of undesirable side-effects. These consist of gastrointestinal disturbances, hypersensitivity reactions, precipitation of urate gravel or calculi in the kidney, or the development of an acute gouty attack [6]. Since salicylates and probenecid are mutually antagonistic in inhibiting urate reabsorption by the tubular epithelium, these two drugs should not be given simultaneously [7].

The most effective means of preventing or reducing the frequency of recurrent attacks of gouty arthritis is the daily administration of combined colchicine and probenecid therapy.

The diet of the gouty patient should consist of restricted purines and fats, approximately 70 gm. of protein daily, and abstinence from beer and wine.

DR. STETTEN: Thank you, Dr. Bunim.

Our own interest in the metabolic defect in

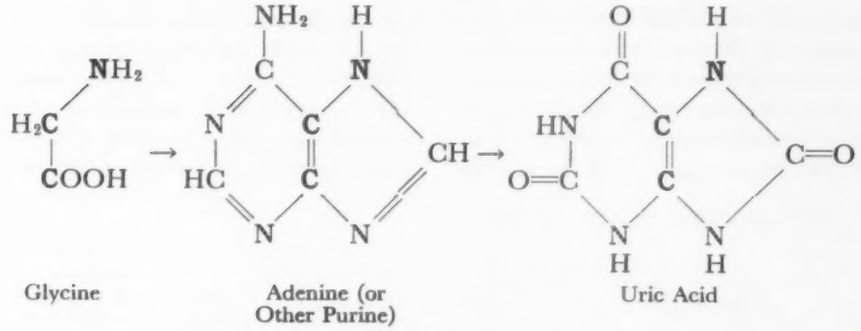


FIG. 5. Contribution of glycine atoms to uric acid.

this disease dates back a matter of eight years and the work which I am now going to discuss very briefly was carried out in collaboration with a number of the members of this program, and in addition, Miss Benedict, Dr. Forsham, Dr. Roche, and Dr. Gutman and Dr. Yü and their collaborators.

The structures of some of the compounds we have under discussion are shown in Figure 5. Glycine has been shown in mammals and birds to be a precursor specifically of two of the carbon atoms and one of the nitrogen atoms of the purine nucleus, ultimately appearing in the uric acid excreted.

Our own studies started with an attempt to measure a quantity which we termed the miscible pool of uric acid [*8*]. To explain the basis for these studies, let the quantity of uric acid which is contained in the body in a miscible state be represented as "A." If this is enriched with isotope to some value, "I," and if one then makes the plausible assumptions that the quantity in the miscible pool is constant, that the rates of ingress and egress from the pool are equal to each other and also constant, one may then show a logarithmic relationship between the isotope abundance and time. The logarithm of the isotope concentration plotted against time will give a straight line with a slope equal to the fraction of the pool replaced per unit of time, and an intercept equal to the logarithm of the isotope concentration at the moment of mixing at time zero. The validity of this derivation is confirmed by experimental results obtained in the normal subject. We have now done quite a number, and similar studies have been carried out in the laboratory of Dr. Talbott [*9,10*].

Essentially linear relationships are secured when the logarithm of the isotope in excreted uric acid is plotted against time after the intravenous injection of uric acid in tracer amounts. In these experiments the urate was labeled with N^{15}; more recently, Dr. Seegmiller has been doing experiments in which C^{14}-labeled urate was administered and essentially identical relationships were observed. Similar linearity of response plotted on these coordinates has also been found in gouty subjects. From the slopes and the intercepts of such plots one may calculate the magnitude and the turnover rate of the miscible pool of urate.

TABLE 1
INJECTION OF ISOTOPIC URIC ACID INTO NORMAL SUBJECTS*

Subject	Miscible Pool (A) (mg.)	Slope (K) (day^{-1})	Turnover (KA) (mg./day)	Urinary Excretion (B) (mg./day)	Surplus (KA-B) (mg./day)	Body Weight (C) (kg.)	Mean Concentration in Body Water (A/7C) (mg. %)	Mean Serum Level (mg. %)
D. C.	1341	0.533	715	602	113	73	2.6	6.0
R. B.	1173	0.591	693	563	130	62	2.7	6.2
G. W.	1145	0.757	867	616	251	76	2.2	4.4

* From Benedict, Forsham and Stetten [*8*].

In Table 1 are values obtained in normal subjects. The magnitude of the miscible pool is computed as the quantity of uric acid which dilutes the isotopically injected uric acid at the moment of injection. It will be noted that the value is in the neighborhood of 1 gm. There is about 1 gm. of uric acid in the body of the normal male in this miscible condition. It turns over fairly rapidly. Fifty to 75 per cent of this pool is replaced daily by newly synthesized uric acid. It was noted early that in every case—and this has been the recurrent experience from a number of laboratories—the quantity of uric acid cal-

culated as entering the miscible pool each day exceeded the quantity of uric acid excreted in the urine. The surplus, which is the difference between these two figures, ranges from 100 to 250 mg. per day. If it is believed, as we believe in these normal subjects, that uric acid is not accumulating, this must represent uric acid which is disposed of by other routes.

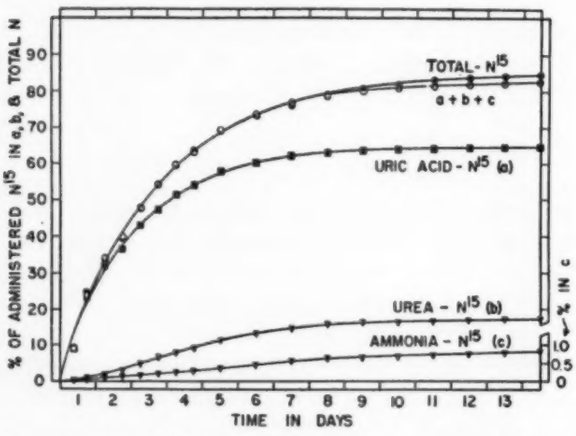


FIG. 6. Cumulative recoveries of N^{15} in major urinary constituents after administration of uric acid N^{15} to a normal subject. The recovery of N^{15} in each constituent expressed cumulatively in per cent of the administered dose has been plotted against time. (From Wyngaarden and Stetten[*11*].

Further to explore this, Dr. Wyngaarden and I—actually he was the subject as well as the chemist in this project—gave rather larger amounts of isotopic uric acid N^{15}, and then isolated, in addition to uric acid, urea and ammonia from the urine. Isotope was found in both urea and ammonia, as well as in uric acid (Fig. 6). This we believe to be the first clear cut demonstration of the occurrence of uricolysis in the normal human male [*11*].

It should be mentioned that this is the rule rather than the exception in the mammalian species, other than the anthropoid apes and the much discussed Dalmatian coach hound. In most mammals that have been studied, uric acid is abundantly destroyed. In man there has been a controversy dating back thirty years as to whether uric acid is or is not destroyed. We take the experiment described as quite conclusive evidence that uric acid is destroyed in man and in Table II are shown some numbers.

Some 17 per cent of all of the isotopic nitrogen administered as uric acid was excreted as urea in the succeeding nine or ten days of the experiment. Because it was known that many bacteria, including some forms which are known to occur in the gastrointestinal tract, are capable of catabolizing uric acid, it was considered important to attempt to ascertain whether this was a mammalian or microbial reaction. Dr. Wyngaarden was placed on a regimen of phthalylsulfathiazole which produced a remarkable bacteriostasis in his intestinal tract. The experiment was then repeated and in the second experiment essentially the same amount of uric acid nitrogen was found to be recoverable in the excreted urea. From this we were forced to believe that the uricolysis that was being observed was a mammalian process. The organic site where this occurred we could not infer, and this has been a matter of debate in various laboratories. It may occur in the bile; it may occur in the white cells; it has been proposed that it might occur in the red cells [*12*]. The answer to this question is not known.

TABLE II
URINARY EXCRETION OF NITROGENOUS CONSTITUENTS IN 14-DAY PERIOD AFTER URIC ACID-N-^{15} ADMINISTRATION TO THE NORMAL SUBJECT*

Substance Isolated	Total Per cent of Administered N^{15}
Uric acid	65.6
Urea	16.8
Ammonia	0.5
Total nitrogen	74.6

* From Wyngaarden and Stetten [*11*].

TABLE III
INJECTION OF ISOTOPIC URIC ACID INTO GOUTY SUBJECTS*

Subject	Miscible Pool (A) (mg.)	Body Weight (C) (kg.)	Mean Concentration in Body Water (A/7C) (mg. %)	Mean Serum Level (mg. %)
B. S.	4742	74	9.2	6.9
A. L.	18450	75	35.1	9.6

* From Benedict, Forsham and Stetten [*8*].

The data presented thus far have related to normal subjects. We have also by this method studied gouty subjects and two experiments are summarized in Table III. The first gouty subject

had a history of two attacks of podagra. His serum urate was not remarkably elevated. However, his miscible pool was four times that observed in the normal subject. The second subject was known as the goutiest man in Boston. His miscible pool was some fifteen times the value observed in the normal. His serum urate was high but certainly not high in proportion to the quantity of the urate which was present in this man's body and capable of prompt mixing with intravenously injected uric acid. This quantity, as a matter of fact, was so large it could not be conveniently accounted for as uric acid in solution. We were forced to conclude that a major portion of his miscible urate resided in the enormous tophaceous masses which this patient presented at a variety of joints.

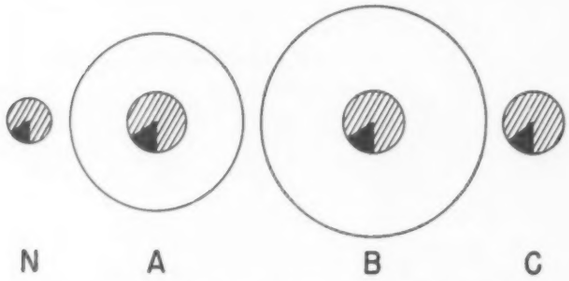


FIG. 7. Distribution of urate miscible pool in ■ plasma, ▨ extravascular water, □ solid phase and miscible pool. A, initial distribution; B, after six months without specific medication; C, after three months therapy with sodium salicylate; N, average values of normal subjects. (From Stetten. *Bull. New York Acad. Med.*, 28: 664, 1952.)

On the basis of rather conventional assumptions, we have divided the urate contained in the miscible pool into three compartments (Fig. 7), the solid black sector representing urate which can be accounted for as in solution in plasma, the hatched sector representing urate which can be accounted for as in solution in extravascular water, and the unshaded area representing urate which is both in the miscible pool and simultaneously in the solid phase. The figure "N" represents the average of a number of normal observations. The figure labeled "A" was our first study on this extremely gouty subject. It should be borne in mind that certainly not all the urate in the tophi was miscible. This man probably had many pounds of uric acid that he was carrying around with him, much of which was undoubtedly immiscible with serum urate.

He was seen some six months later. During this interval from "A" to "B" there was no specific therapy and at this time his miscible pool had increased from some 18,000 mg. to a matter of 30,000 mg. He was then given uricosuric doses of salicylate and fortunately he was one of those patients who could tolerate large amounts of salicylates for a long period of time. Some three months later he was seen again, and at this time the magnitude of his miscible pool had declined from 30,000 mg. to a matter of 2,000 mg. or roughly twice normal. At this time when we computed the quantity of still miscible urate that was in the solid phase, it turned out to be essentially zero.

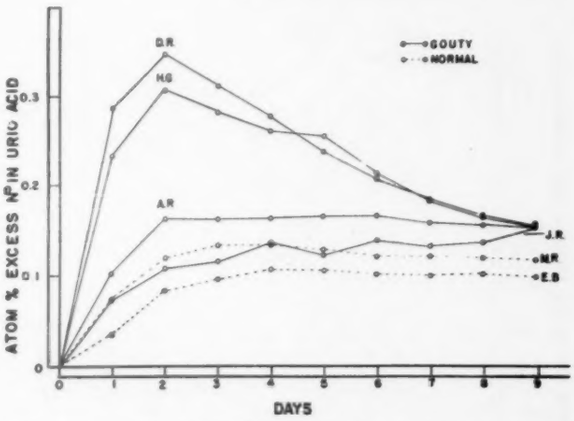


FIG. 8. Incorporation of glycine N^{15} into urinary uric acid. (From Benedict, Yü, Bien, Gutman and Stetten. *J. Clin. Investigation*, 32: 775, 1953.)

We were then faced with the problem that in gout there is far more miscible urate than there is in the normal subject. The question is: Where did it come from? Two possibilities had to be considered from the metabolic point of view: One, that it was over-produced; the other, that it was disposed of less rapidly than was normal. In order to investigate this it was convenient and desirable to give a precursor of uric acid rather than uric acid itself.

Figure 5 shows the relationship between glycine and uric acid. In these experiments the N^{15} label was used. More recently, C^{14}-labeled glycine was used and again the results are roughly comparable.

Figure 8 shows results of a number of experiments in which the abundance of N^{15} in urinary uric acid is plotted as a function of time after the ingestion of glycine N^{15}. The broken lines represent normal subjects, and it will be observed that in the normal subjects the abundance of isotope in urine rises to a maximum three or four days after its administration. Similar curves have

been achieved on at least two gouty patients in this study. However, there have been a number of gouty patients—two represented here—in whom something quite different has happened. The abundance of N^{15} rose rapidly to a maximum which was achieved in these two cases on the second day, followed thereafter by a decline which is far more rapid than normal.

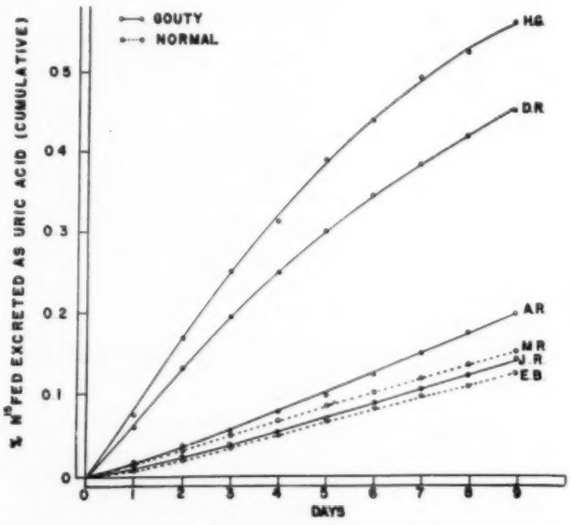


FIG. 9. Cumulative values for incorporation of N^{15} from glycine into uric acid in the same patients as in Figure 8. (From Benedict, Yü, Bien, Gutman and Stetten. *J. Clin. Investigation*, 32: 775, 1953.)

These same values plotted cumulatively up to the ninth day are shown in Figure 9. It will be observed that urate is a relatively minor product of glycine nitrogen. In the normal patients only about one-tenth of one per cent of all of the glycine ingested could be accounted for as uric acid. This is true, as will be seen, of some of the gouty patients but in these two it will be noted that the value is some three or four times the normal.

We observed that there was very good correlation between the ability of the patient to convert ingested glycine into uric acid and the abundance of uric acid excreted daily while on a basal diet. There are some gouty patients, according to Gutman's figures about 25 per cent, who, on a basal diet, excrete more uric acid than normal. It appears to be among them that we find patients who do this sort of thing. Whether this represents a different disease than that seen in the other subjects, or whether these two pictures represent different phases of the same disease, is at the present time not entirely clear.

In Figure 10 are shown metabolic pathways to indicate that in certain gouty subjects glycine appears to find its way into uric acid by a rapid route which appears to circumvent the metabolically sluggish intermediates, particularly the purines adenine and guanine, which are known to be relatively inert metabolically, and particularly in the deoxyribonucleic acids of the adult cell.

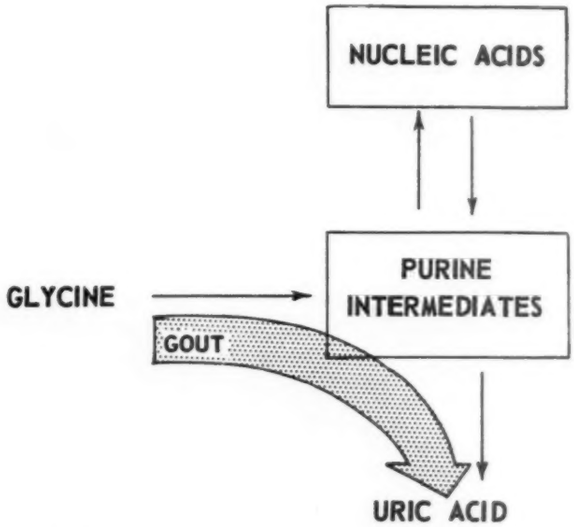


FIG. 10. Proposed metabolic shunt in certain patients with gout.

It is suggested there might be operative a mechanism which is perhaps not dissimilar to the mechanism normally operative in the bird and reptile. In these species uric acid is the major excretory form of metabolic nitrogen. The bird makes an abundance of uric acid from a variety of nitrogenous precursors and excretes them. However, it is endowed with a kidney capable of excreting urine, as is known to you, which is generally solid or semi-solid. We are inclined to picture the gouty individual as having at least a vestige of avian metabolic pathways without being simultaneously endowed with avian kidneys.

The next component that we thought should be investigated was the pattern of amino-imidazolecarboxamide, and I shall ask Dr. Seegmiller to report on this compound.

DR. J. E. SEEGMILLER: For further exploration of the metabolic abnormalities in gout we chose to study the pattern of incorporation of 4-amino-5-imidazolecarboxamide (referred to as AIC) into uric acid. Although this compound was not known to be incorporated into uric acid in man, previous studies in the rat had demonstrated its

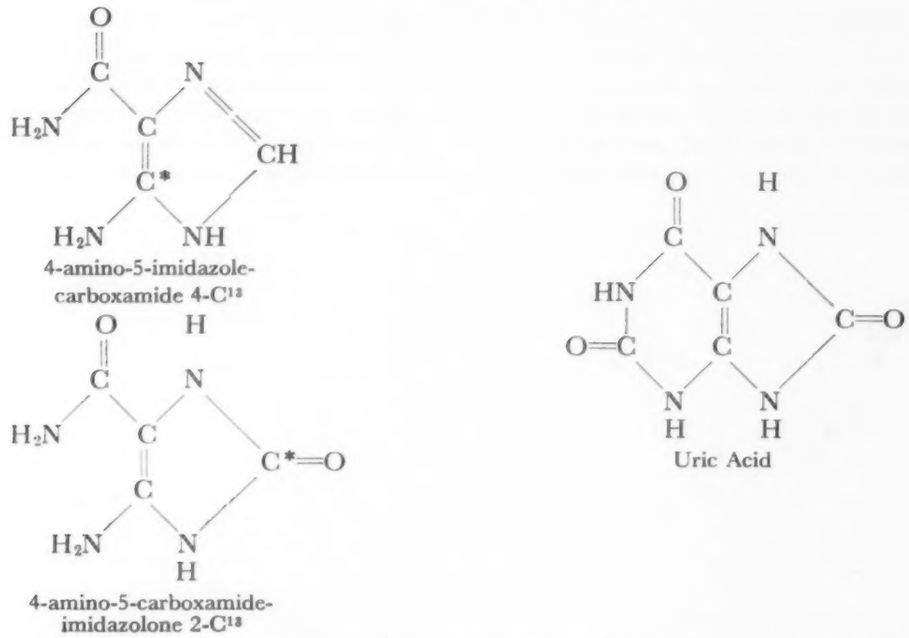


FIG. 11. Structural relationship of 4-amino-5-imidazolecarboxamide and 4-amino-5-carboxamideimidazalone to uric acid.

incorporation into the counterpart of uric acid, allantoin [*13*], as well as into tissue purines [*14*].

The close relationship of the chemical structure of this compound to purines is apparent in Figure 11. We were able to synthesize this compound in Dr. Stetten's laboratory with carbon 13, the heavy, non-radioactive isotope of carbon, in position 4 as indicated. Another compound, 4-amino-5-carboxamide-imidazalone, also shown in Figure 11, is structurally more closely related to uric acid than is AIC. It seemed reasonable to think that a pathway of urate biosynthesis that would by-pass the usual purine metabolism might very well involve a compound such as this. It was therefore synthesized with carbon 13 in the two-position, as indicated, by Dr. Lloyd H. Smith, also in Dr. Stetten's laboratory. Dr. Smith found that the imidazalone was not incorporated into uric acid by either the pigeon or normal man [*15*]. AIC, on the other hand, was found to be a very effective precursor of uric acid in man.

Dr. Laster and I first determined the pattern of incorporation of this compound into urinary uric acid in two normal human subjects [*16*], maintained on a 70 gm. protein diet, constant in calories, and very poor in exogenous purines. After an equilibration period of at least ten days AIC was given orally with breakfast. Since we were also interested in doing this in gouty patients and would need to classify them as to their pattern of incorporation of glycine N^{15}, this substance was given simultaneously.

In Table IV we see that 20 per cent of the ingested dose of AIC was excreted in the urine,

TABLE IV

RECOVERY OF C^{13} FROM AIC IN URINARY PRODUCTS*

(The Cumulative Recoveries of C^{13} in Urinary AIC and Uric Acid have been Calculated as Percentages of C^{13} fed as AIC)

	Per cent of Administered Dose of AIC	
	J. E. S.	L. L.
C^{13} in AIC excreted	20	21
C^{13} in uric acid excreted in 14 days	21	20
C^{13} in uric acid excreted in 15–21 days	..	3
Total C^{13} accounted for	41	44

* From Seegmiller, Laster and Stetten [*16*].

unchanged, within the first eight hours. Another 20 to 23 per cent of the carbon 13 was recovered in the urinary uric acid during the succeeding two to three weeks. This leaves around 56 to 59 per cent of the AIC unaccounted for. A portion

of this is presumed to have entered tissue purines and possibly other metabolites.

The AIC in the urine was isolated and the isotope content was found to be essentially the same as the dose administered, indicating that there had been no dilution by a body pool of the

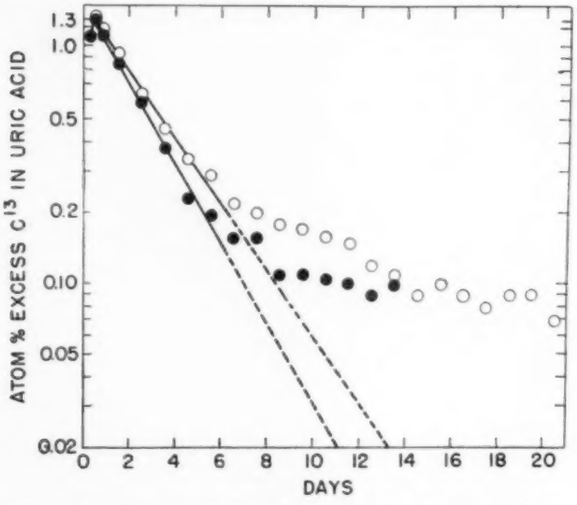


FIG. 12. Incorporation of 4-amino-5-imidazolecarboxamide-4-6^{13} (AIC) into urinary uric acid in two normal subjects. (From Seegmiller, Laster and Stetten[*16*]).

substance. There was no evidence, then, that AIC is a normal metabolic intermediate. Evidence from other sources suggests that the ribotide of AIC is the normal intermediate in purine synthesis [*17*]. Although AIC is probably not a normal intermediate, it is very promptly converted to uric acid. (Fig. 12.) During the two days following its ingestion, 200 mg. of extra uric acid appeared in the urine. The maximum isotope incorporation of 1.3 atom per cent excess was achieved at fourteen hours, and corresponds to an isotope dilution of only 8.5 fold. This prompt incorporation can be contrasted to the rather slow incorporation of glycine which is maximal only after three or four days, indicating that AIC entered the metabolic chain of reactions, as we had anticipated, at a point closer to uric acid. The decline in carbon 13 enrichment has a rapid and a slow component when plotted on hemilogarithmic coordinates.

The rapid component probably results primarily from the turnover of the body pool of uric acid. If this were the only labeling that had occurred we would expect it to follow a straight line. However, after the sixth day a slower contribution of carbon 13 to the uric acid pool was apparent. Presumably this represents a turnover of uric acid precursors which had been labeled by the administration of AIC-C^{13}. That tissue purines may be among these precursors seems reasonable, as the animal studies showed that nucleic acid purines are labeled by AIC.

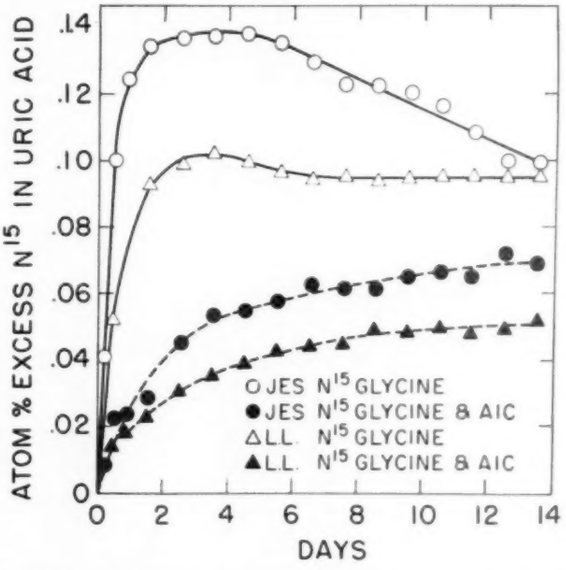


FIG. 13. Decreased incorporation of glycine N^{15} into urinary uric acid when administered with AIC to a normal subject.

An unexpected finding was a marked depression of the enrichment of uric acid with respect to N^{15} below the values expected from previous glycine N^{15} studies of normal subjects, as shown in Figure 13. That this was an effect of AIC, was shown by repeating the studies, using only glycine N^{15} in the same subjects. The results are shown by the upper lines. Evidence that this was not the result of simple dilution with the extra uric acid arising from AIC was provided by degrading the uric acid molecule and determining the N^{15} content in various positions. That this was not an effect of the bodies' disposition of glycine was shown by obtaining essentially identical patterns of N^{15} incorporation into urinary creatinine in the presence and absence of AIC, showing that the glycine fed was metabolically available for creatinine synthesis. This evidence supports the view that this is a true suppression of the *de novo* synthesis of uric acid, and presumably of purine, by a pharmacologic action of AIC.

These studies were repeated in several gouty subjects (Fig. 14), and AIC again exerted a similar depression of N^{15} incorporation from

glycine into uric acid. When glycine N^{15} was given alone the pattern of incorporation was essentially that of the normal individual, so this patient was not one who incorporates excessive amounts of N^{15} and presumably therefore does not have the metabolic shunt from glycine.

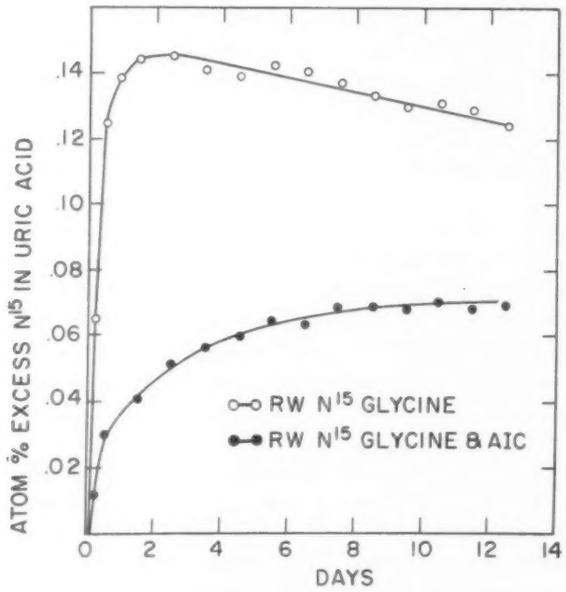


FIG. 14. Decreased incorporation of glycine N^{15} into urinary uric acid when administered with AIC to a patient with gout.

In Figure 15 is shown the pattern of incorporation of AIC into uric acid in a gouty subject, indicated by the upper line. The maximum C^{13} incorporation was fairly close to the values obtained in the normal individual. Uric acid C^{14} was injected at the time of maximum enrichment with C^{13}. The subsequent decline in C^{14} enrichment, shown by the lower line, should then be indicative of the pool turnover of urate. The fact that these lines do not coincide graphically, illustrates the presence of an initial rapid contribution to the urate pool, followed by a slow contribution of C^{13} to uric acid; hence, there are two pathways to uric acid from AIC. When one considers that this patient's miscible pool of uric acid was around 2,000 mg., which is about twice normal, while the maximum enrichment with C^{13} was close to values seen in the normal subject, this would suggest a greater total initial incorporation in these patients. A greater per cent of C^{13}, fed as AIC, appeared in urinary uric acid in the ensuing three weeks in all three gouty patients than in normal subjects, but the differences between some of them is not great.

In summary we have shown the following: (1) AIC is incorporated into uric acid in man via two pathways—a rapid, direct pathway and a slower, indirect path, possibly via body purines. (2) AIC administration at the dosage used interferes with the synthesis of uric acid from

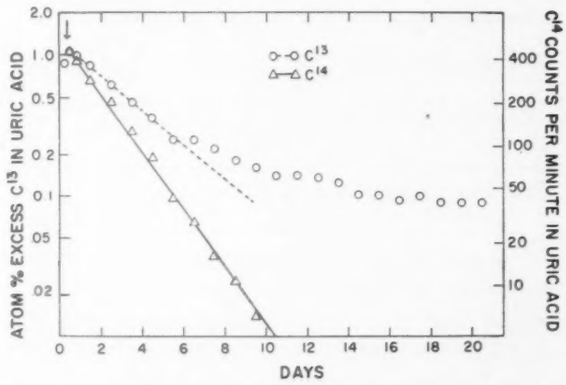


FIG. 15. Incorporation of 4-amino-5-imidazolacarboxamide-4-C^{13} (AIC) into urinary uric acid in a patient with gout. Urate pool was labeled with uric acid -2-C^{14} injected intravenously at time indicated by the arrow.

glycine. (3) There is some preliminary evidence suggesting that a greater amount of C^{13} is incorporated into urinary uric acid of three gouty patients fed AIC-C^{13} than was found in normal individuals, although many aspects of the pattern of incorporation were very similar to that of the normal.

DR. STETTEN: Thank you, Dr. Seegmiller. Dr. Wyngaarden will now report on the studies that have been conducted of the effects on uric acid metabolism of the several uricosuric agents that are in use today.

DR. JAMES B. WYNGAARDEN: Uric acid exists in plasma at pH 7.4 almost exclusively as the monoalkali salt. Plasma urate is completely ultrafiltrable [*18*], and dialysis equilibration studies have indicated that something less than 4 per cent of plasma urate exists in bound form [*19*]. For these reasons plasma urate is believed to be completely filterable at the glomerulus.

In normal man all but 5 to 10 per cent of the filtered urate is reabsorbed in the renal tubules [*20,21*]. Studies conducted on gouty individuals in various stages of their disease have indicated there is no important departure from this pattern in gout [*22,23*]. Despite marked variations in renal function in this disease, and therefore in the filtered load of urate, all but 5 to 10 per cent of that filtered load is reabsorbed.

TABLE V

EFFECT OF PHENYLBUTAZONE (PB) ON URATE METABOLISM IN TWO NORMAL SUBJECTS*

Subject	Experiment	Serum Urate (mg. %)	Miscible Pool (A) (mg.)	Turnover (KA) (mg./day)	Urinary Excretion (B) (mg./day)	B/KA × 100 (%)	Recovery of Injected N^{15} Uric Acid (%)	Volume of Distribution of A (L.)
J. W.	Control	5.4	1272 ± 58	580 ± 28	476 ± 7	82	76	23.6
	PB	2.5	716 ± 108	776 ± 122	644 ± 11	83	93	28.6
S. N.	Control	4.3	651 ± 34	501 ± 27	400 ±13	80	73	15.1
	PB	1.7	341 ± 32	413 ± 39	405 ± 5	98	93	20.0

* From Wyngaarden [*28*].

It is now known that young patients with gout frequently have normal urate clearances [*24*]. It follows that in such individuals with high serum urate levels and normal glomerular filtration rates, both the total quantity of urate reabsorbed and the total quantity of urate excreted daily are in excess of normal. However, many patients with long-standing gout excrete less than normal quantities of urate daily. Because of this observation many of the older treatises on gout attributed the accumulation of urate by the gouty individual to a specific renal defect in urate excretion. However, application of the most refined available methods for assessing discrete renal functions has failed to establish a primary intrinsic defect in renal excretion of urate in gout [*22,23*]. The low urinary urate excretion commonly found in late gout is attributable to impairment of renal function which so often develops as a complication of this disease.

In certain disorders in which there is widespread tubular damage, such as Wilson's disease [*25*] or de Toni-Fanconi syndrome, a considerably smaller percentage of filtered urate may be reabsorbed. In one patient with Wilson's disease recently studied here, 30 per cent of the filtered urate appeared in the urine [*26*]. In one case of de Toni-Fanconi syndrome studied by Sirota et al. [*22*], the filtered urate appeared quantitatively in the urine. Obviously, such renal defects are reflected in abnormally low urate levels.

The uricosuric agents of use in gout have been found to act by suppressing partially the tubular reabsorption of filtered urate. This has been shown to be true of probenecid [*22*], of salicylates [*27*], of phenylbutazone [*28*], and of adrenalcortical steroid hormones [*29*].

When phenylbutazone was first used it was reported from a number of centers [*30–32*] that this drug lowered the serum urate level in gouty subjects without causing an increase in urinary urate excretion. Because of these findings it was suggested that phenylbutazone might influence the production [*32*], distribution or destruction of uric acid in the body.

We undertook to investigate this problem with the use of N^{15}-labeled uric acid. Two individuals were placed on purine-poor diets and urinary and serum urate levels were determined for several days. When urinary excretion values had reached constant levels a small quantity of uric acid N^{15} was injected and the miscible pool and the rate of turnover of uric acid determined. Following this, phenylbutazone was administered for several days and after a suitable uricosuric effect was achieved the N^{15} uric acid study was repeated. The recovery of unchanged isotopic uric acid in urine was determined for both experiments; also, the apparent volume of distribution of the miscible uric acid was calculated for both experiments. (Table v.)

In the first subject the miscible pool of uric acid was 1,272 mg. initially. After five days of phenylbutazone ingestion (Figure 16), the plasma urate had declined from 5.4 to 2.0 mg. per cent, and the pool was 716 mg., a value 556 mg. less than the initial value of the miscible pool. During this time there had been a cumulative increment in urinary urate excretion of 598 mg. Therefore, the decline of the uric pool size was quantitatively explained by the increment in urinary urate excretion. Furthermore, the turnover of urate, that is, the rate of generation of new urate molecules, was not significantly different prior to and during phenylbutazone ingestion.

The portion of injected isotopic uric acid re-

covered unchanged in urine in the initial experiment was 76 per cent and in the subsequent study 93 per cent, so there appeared to be no increase in the quantity of uric acid disposed of by extra-renal pathways or by uricolysis during phenylbutazone ingestion. The volume of distribution of miscible urate was 24 L. in the initial experiment and 29 L. in the subsequent study. This increase was not sufficient to account for the very large decline in the serum urate level. However, Yü and Gutman have recently shown that at low doses phenylbutazone is not uricosuric and may cause sufficient fluid retention to lead to a dilution of plasma urate [*33*].

In the second subject similar results were obtained. (Table v.) In this subject the miscible pool declined 310 mg. during a period in which an excess of 359 mg. of urate was excreted. Again there was no evidence for a significant effect of the drug upon the rate of urate synthesis and no evidence that extra-renal disposal of urate had been augmented. An increase in the volume of distribution of the miscible urate was again noted, and in this subject could be attributed to fluid retention. On the basis of these studies it can be stated that phenylbutazone in large doses acts at the renal level to augment urate excretion, and has no important effect on the rates of synthesis, degradation or extra-renal disposal of urate. The studies of Yü, Sirota and Gutman [*34*] clearly show that the renal effect is mediated at the tubular level.

One other point deserves mention. When the serum urate level was maximally lowered in both subjects the urinary urate excretion returned toward or to the control levels. In reports from certain laboratories this phenomenon has been interpreted as the development of resistance to the effect of the uricosuric agent. However, if one analyses these results in terms of urate clearance one can readily see that an excretion of 400 mg. per day with a serum level of 2 mg. per cent represents a considerably greater urate clearance than the same excretion with a serum level of 5 mg. per cent. Thus the effect of the drug has not worn off; the suppression of tubular reabsorption of urate is still very much in evidence.

There are situations, however, in which patients respond poorly to these agents, as in patients with severe renal damage as a consequence of long-standing gout. We have studied a few such patients who respond poorly or scarcely at all to probenecid and have found, in agreement with Yü and Gutman [*35*], that in the presence of severe renal damage salicylates are frequently the most effective uricosuric agent. For example, in one subject studied who basally excreted only 250 mg. of urate daily, the urate excretion was increased to 800 or 900 mg. per

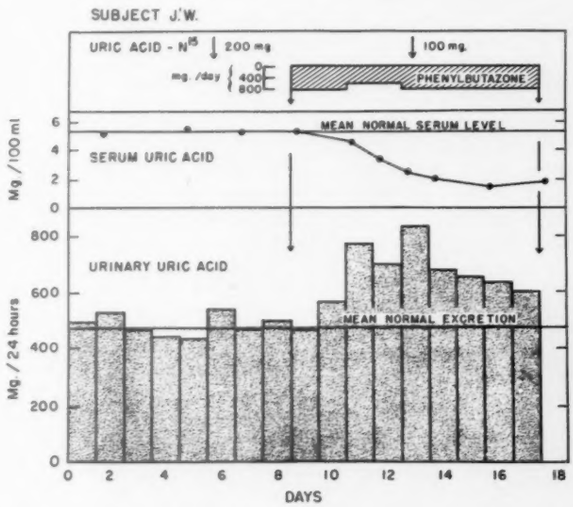


FIG. 16. The effects of phenylbutazone on uric acid excretion. (From Wyngaarden[*28*].)

day by means of salicylates in 4 to 5 gm. daily dosage whereas after administration of 2.0 gm. of probenecid per day urate excretion was maximally 500 mg. daily.

In collaboration with Dr. Seegmiller and Dr. Laster [*36*], we have recently conducted a series of urate clearance studies on two patients with gout and moderately severe renal involvement. In both subjects the inulin and urate clearances were reduced considerably below normal. The fraction of the filtered urate load that was reabsorbed by the tubules was, however, normal. (Table VI.) In both subjects the administration of 4.8 gm. per day of sodium salicylate orally had a greater suppressive effect upon tubular reabsorption of urate than did the administration of 1.0 gm. of probenecid orally, although the response to probenecid, in terms of the increase of C urate/C inulin ratio, was within the usual range [*22*]. The addition of meticorten produced no change in the response to probenecid, nor did meticorten alone, in the dosage administered, have any significant effect upon any of the renal functions measured. These studies suggest that it is not a lack of responsiveness at the renal tubular level which limits the usefulness of probenecid in gouty patients with severe renal disease but rather that the reduction in glomerular filtra-

TABLE VI

RENAL EFFECTS OF URICOSURIC AGENTS IN GOUTY PATIENTS WITH RENAL DAMAGE

Patient* Age and Sex	Drug Regimen	C_{INULIN}	C_{PAH}	C_{URATE}	$C\frac{UR}{IN}\cdot 100$	$C\frac{IN}{PAH}\cdot 100$
B. T., 50, F	None	30.7†	199	3.27	10.6	16.1
	Sodium salicylate 4.8 gm./d × 6 d	29.2	143	11.89	40.6	20.3
	Probenecid 1.0 gm./d × 2 d	30.8	106	7.77	25.3	29.1
	Probenecid 1 gm./d + Meticorten 30 mg./d × 3 d	30.1	113	7.50	24.9	26.7
	Meticorten 30 mg./d × 2 d	29.2	157	4.17	14.3	18.6
	None	32.9	146	4.00	12.2	22.5
J. D., 52, M	None	56.6	276	4.80	8.4	20.5
	Sodium salicylate 4.8 gm./d × 8 d	44.5	275	17.7	39.8	16.2
	Probenecid 1.0 gm./d × 8 d	57.5	257	10.0	17.5	23.0
	Probenecid 1.0 gm./d + Meticorten 30 mg./d × 7 d	57.8	257	8.5	14.7	22.5
	Meticorten 30 mg./d × 3 d	59.6	268	3.8	6.2	20.8

* Patient B. T. was a fifty year old woman with chronic non-tophaceous gout of eight months' duration; serum urate 10 mg. per 100 ml. Patient J. D. was a fifty-two year old man with severe chronic tophaceous gout of twenty years' duration; serum urate 12.2 mg. per cent.

† All values are averages of three or more clearance periods, ranging from twenty to ninety minutes. Urine was collected by spontaneous voidings, with a water load of 240 ml. each half hour before and during the clearance period.

tion rate so limits the presentation of urate to the tubules that only a modest uricosuric response is possible. These studies corroborate observations of others that salicylates in adequate doses are effective uricosuric agents, and suggest that these agents may be superior to probenecid in gouty patients with severe renal disease where a drug with greater intrinsic uricosuric effect than probenecid is needed in order to achieve a satisfactory augmentation of urate excretion. Meticorten appears to have no significant renal effect on urate metabolism under the conditions of this study.

DR. STETTEN: Thank you, Dr. Wyngaarden. We have here a disease in which there are two aspects, as Dr. Bunim has mentioned. There is an acute inflammatory disease of the joints. There is a disturbance in purine metabolism. One of the puzzling aspects to me about this disease is the fact that these two phases of the disease apparently are dissociable. Dr. Bunim has already mentioned that colchicine, which has a long and respectable history as the drug of choice in the treatment of the acute disease, has, so far as is known today, no effect upon any aspect of purine metabolism as yet elucidated. Similarly, the administration of uricosuric drugs has no therapeutic effect upon the attacks of acute arthralgia. Indeed, in the case presented today one of the attacks of acute arthritis occurred during a successful regimen of uricosuria. This has been a general experience.

In addition to the therapeutic dissociation of these two phases of the disease, the natural occurrence of the one without the other is noteworthy. As Dr. Bunim mentioned, frequently hyperuricemia is seen in the relatives of gouty patients without any clinical evidence of gouty arthritis. Conversely, cases have been reported of colchicine-responsive arthritis which clini-

cally resembles gout in the absence of any hyperuricemia.

One wonders, indeed, whether or not these two aspects of the disease are causally related at all—whether perhaps they may not be genetically related.

SUMMARY

The clinical aspects of gout and its management have been reviewed as they relate to a case of severe tophaceous gout in a young man twenty-two years of age. The early onset at age sixteen, the rapid development of tophi and difficulty in obtaining adequate uricosuria were unusual aspects of this case.

The histopathology of gout has been reviewed and evidence presented that complex matrix materials are associated with urate crystals at some stages in the evolution of the tophus.

A summary has been presented of our current knowledge of purine metabolism in the normal and gouty human as revealed by the use of uric acid N^{15} and the isotopically labeled purine precursors, glycine N^{15} and 4-amino-5-imidazole carboxamide-4-C^{13} (AIC).

The effect of phenylbutazone on urate metabolism has been investigated with the aid of uric acid N^{15}. Evidence is presented to show that it increases urate excretion, thus decreasing the miscible urate pool without appreciably affecting the rate of urate synthesis, degradation or extrarenal disposal.

REFERENCES

1. Garrod, A. B. The Nature and Treatment of Gout and Rheumatic Gout. London, 1859. Walton & Maberly.
2. Talbott, J. H. Gout and Gouty Arthritis. Philadelphia, 1953. Grune & Stratton.
3. Smyth, C. J. and Huffman, E. R. Gouty arthritis—diagnosis and treatment. *M. Clin. North America*, 39: 543, 1955.
4. Modern, F. W. S. and Meister, L. The kidney of gout, a clinical entity. *M. Clin. North America*, 36: 941, 1952.
5. Gutman, A. B. and Yü, T. F. Prevention and treatment of chronic gouty arthritis. *J. A. M. A.*, 157: 1096, 1955.
6. Boger, W. P. and Strickland, S. C. Probenecid (benemid)—its uses and side-effects in 2,502 patients. *Arch. Int. Med.*, 95: 83, 1955.
7. Pascale, L. R., Dubin, A., Bronsky, D. and Hoffman, W. S. Inhibition of the uricosuric action of benemid by salicylate. *J. Lab. & Clin. Med.*, 45: 771, 1955.
8. Benedict, J. D., Forsham, P. H. and Stetten, DeW., Jr. The metabolism of uric acid in the normal and gouty human studied with the aid of isotopic uric acid. *J. Biol. Chem.*, 181: 183, 1949.
9. Bishop, C., Garner, W. and Talbott, J. H. Pool size, turnover rate, and rapidity of equilibration of injected isotopic uric acid in normal and pathological subjects. *J. Clin. Investigation*, 30: 879, 1951.
10. Buzard, J., Bishop, C. and Talbott, J. H. Fate of uric acid in normal and gouty human. *J. Chron. Dis.*, 2: 42, 1955.
11. Wyngaarden, J. B. and Stetten, DeW., Jr. Uricolysis in normal man. *J. Biol. Chem.*, 203: 9, 1953.
12. Bien, E. J. and Zucker, M. Uricolysis in normal and gouty individuals. *Ann. Rheum. Dis.*, 14: 409, 1955.
13. Miller, C. S., Gurin, S. and Wilson, D. W. C^{14} labeled 4(5)-amino-5(4)-imidazolecarboxamide in the biosynthesis of purines. *Science*, 112: 654, 1950.
14. Conzelman, G. M., Jr., Mandel, H. G. and Smith, P. K. The incorporation of radiocarbon from 4-amino-5-imidazolecarboxamide into the purines of tumor-bearing mice. *J. Biol. Chem.*, 201: 329, 1953.
15. Smith, L. H., Jr. and Yates, P. The synthesis of 4-amino-2(3H)-oxo-5-imidazolecarboxamide. *J. Am. Chem. Soc.*, 76: 6080, 1954.
16. Seegmiller, J. E., Laster, L. and Stetten, DeW., Jr. Incorporation of 4-amino-5-imidazolecarboxamide-4-C^{13} into uric acid in the normal human. *J. Biol. Chem.*, 216: 653, 1955.
17. Buchanan, J. M. and Schulman, M. P. Biosynthesis of the purines. III. Reactions of formate and inosinic acid and an effect of the citrovorum factor. *J. Biol. Chem.*, 202: 241, 1953.
18. Yü, T. F. and Gutman, A. B. Ultrafiltrability of plasma urate in man. *Proc. Soc. Exper. Biol. & Med.*, 84: 21, 1953.
19. Wyngaarden, J. B. Uric acid. In: Cyclopedia of Medicine, Surgery, Specialities, vol. 6, p. 344. Philadelphia, 1955. F. A. Davis Co.
20. Berliner, R. W., Hilton, J. G., Yü, T. F. and Kennedy, T. J., Jr. The renal mechanism for urate excretion in man. *J. Clin. Investigation*, 29: 396, 1950.
21. Smith, H. W. The Kidney. New York, 1951. Oxford University Press.
22. Sirota, J. H., Yü, T. F. and Gutman, A. B. Effect of benemid (p-di-n-propylsulfamyl-benzoic acid) on urate clearance and other discrete renal functions in gouty subjects. *J. Clin. Investigation*, 31: 692, 1952.
23. Talbott, J. H. Gout. In: Christian, H. A. The Oxford Medicine, vol. 4, p. 79. New York, 1943. Oxford University Press.
24. Friedman, M. and Byers, S. O. Increased renal excretion of urate in young patients with gout. *Am. J. Med.*, 9: 31, 1950.
25. Bishop, C., Zimdahl, W. T. and Talbott, J. H. Uric acid in two patients with Wilson's disease (hepatolenticular degeneration). *Proc. Soc. Exper. Biol. & Med.*, 86: 440, 1954.
26. Wyngaarden, J. B. and Seven, M. Unpublished data.
27. Yü, T. F., Sirota, J. H. and Gutman, A. B. Influence of plasma salicylate concentration on urate clearance in man. *Clin. Research Proc.*, 1: 107, 1953.
28. Wyngaarden, J. B. The effect of phenylbutazone on uric acid metabolism in two normal subjects. *J. Clin. Investigation*, 34: 256, 1955.

29. Ingbar, S. H., Kass, E. H., Burnett, C. H., Relman, A. S., Burrows, B. A. and Sisson, J. H. The effects of ACTH and cortisone on the renal tubular transport of uric acid, phosphorus, and electrolytes in patients with normal renal and adrenal function. *J. Lab. & Clin. Med.*, 38: 533, 1951.

30. Kuzell, W. C. and Schaffarzick, R. W. Phenylbutazone (butazolidin) and butapryin. A study of clinical effects in arthritis and gout. *California Med.*, 77: 319, 1952.

31. Kidd, E. G., Boyce, K. C. and Freyberg, R. H. Clinical studies of phenylbutazone (butazoldin) and butapyrin (irgapyrin) in rheumatoid arthritis, rheumatoid spondylitis and gout. *Ann. Rheumat. Dis.*, 12: 20, 1953.

32. Johnson, H. P., Jr., Engleman, E. P., Forsham, P. H., Krupp, M. A., Green, T. W. and Goldfien, A. Effects of phenylbutazone in gout. *New England J. Med.*, 250: 665, 1954.

33. Yü, T. F. and Gutman, A. B. Paradoxical retention of uric acid by uricosuric drugs in low dosage. *Proc. Soc. Exper. Biol. & Med.*, 90: 542, 1955.

34. Yü, T. F., Sirota, J. H. and Gutman, A. B. Effect of phenylbutazone (3,5-dioxo-1,2-diphenyl-4-n-butylprazoldine) on renal clearance of urate and other discrete renal functions in gouty subjects. *J. Clin. Investigation*, 32: 1121, 1953.

35. Yü, T. F. and Gutman, A. B. Mobilization of gouty tophi by protracted use of uricosuric agents. *Am. J. Med.*, 11: 765, 1951.

36. Wyngaarden, J. B., Seegmiller, J. E. and Laster, L. Unpublished observations.

Case Reports

Isolated ("Primary") Chylopericardium*

Due to Anomalous Communications with the Thoracic Duct, of Unknown Causation

WILLIAM M. MADISON, JR., M.D.† *and* BRUCE LOGUE, M.D.
Emory University, Georgia

THE effusion of chyle into the pericardial sac in association with massive chylothorax has been the subject of several case reports [*1–3*], but only one case of primary chylopericardium has been reported previously [*4*]. In this case exploratory thoracotomy was performed and a superior mediastinal hygroma (lymphangiomatous hamartoma) was discovered. An anatomic communication had been demonstrated by lipophilic dye studies preoperatively, but no direct communication linking the thoracic duct or the hygroma and the pericardial sac was identified at operation. A large portion of the cystic tumor was removed, followed by ligation of the thoracic duct low in the chest and the establishment of a pericardial window for drainage into the right pleural space. There was no reaccumulation of the chylous effusion in either the pericardial or pleural space following operation [*5*].

An opportunity to study this extremely rare condition was afforded by the discovery of a patient with a chylous pericardial effusion. Using the technic gained from the original case report, a plan of diagnosis and treatment was evolved. These experiences form the content of this report.

CASE REPORT

M. B. G., a forty year old married housewife, was first seen in the Emory University Clinic on June 15, 1955. Seven years previously, in 1948, cardiomegaly had been noted on a routine tuberculosis survey film. Previous chest roentgenograms had not been taken. At this time she gave a seven-year history of mild exertional dyspnea which did not limit normal activity. All other cardiac symptoms were denied. For three years prior to 1955 she had noted several episodes of nocturnal "smothering" relieved by sitting upright in bed. Wheezing respirations were denied. During the previous month mild dependent edema was noticed in the latter part of the day. The weight had been stable for several years.

The past history was not remarkable. She had undergone two normal pregnancies, but the first child died shortly after birth for an unknown reason. The family history was unrevealing. At the age of seventeen she had sustained contusions of the forehead and both thighs in an automobile accident. Chest injuries and falls were denied.

Physical examination disclosed a plump white woman appearing quite healthy. The temperature was 98.6°F. The respirations were 14 per minute and normal in depth. The pulse was 72, strong and regular; the blood pressure was 130/70 mm. Hg. The cervical veins were flat and no unusual pulsations were noted in the neck. On cardiac examination the apical impulse was not palpable and cardiac dullness extended to the mid-axilla. The heart sounds were normal and no murmurs were heard. No enlargement of the liver or spleen was detected and neither ascites nor dependent edema was present. The remainder of the physical examination was normal. An electrocardiogram showed a normal mean QRS axis, sinus rhythm and low voltage. (Fig. 1.) Fluoroscopic examination revealed generalized enlargement of the cardiac silhouette and pulsations were of markedly diminished amplitude. No undue prominence of the hilar vessels was seen and a barium swallow was normal. Circulation time, arm to tongue (decholin®) was twelve seconds; the venous pressure was 110 mm. saline solution.

Laboratory examination revealed a normal urinaly-

* From the Emory University Hospital and the Department of Medicine, Emory University School of Medicine, Emory University, Georgia.
† Trainee, National Heart Institute.

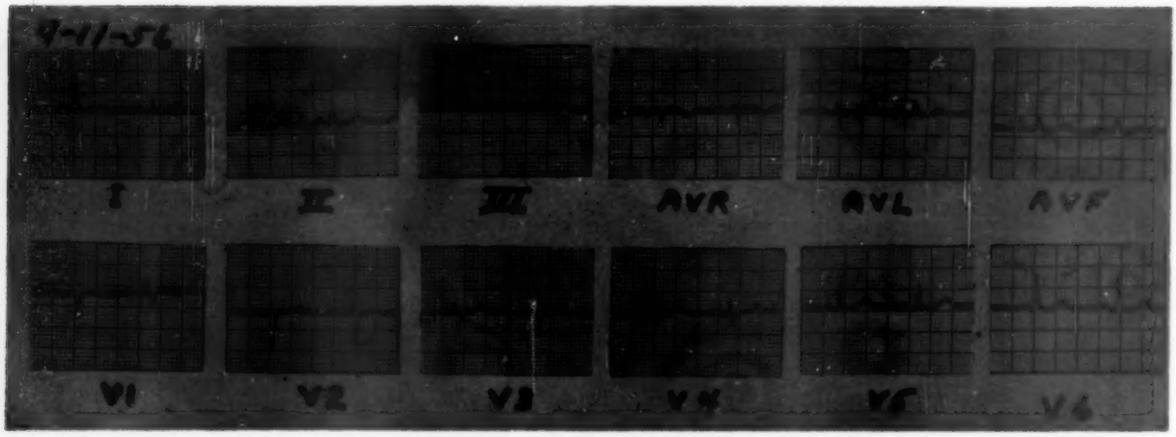


FIG. 1. Preoperative electrocardiogram showing low voltage. (1 cm. = 1 mv.).

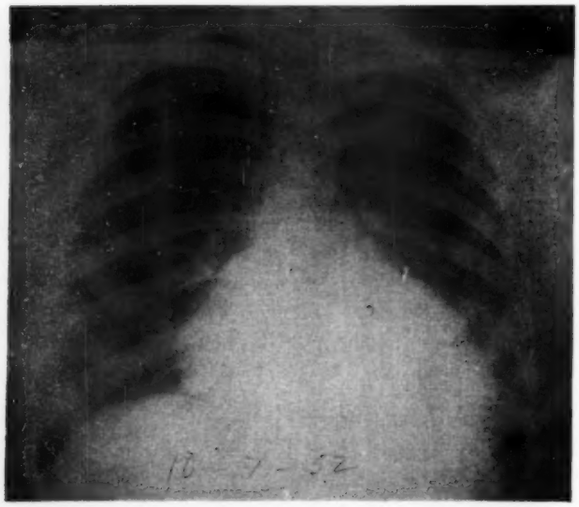


FIG. 2A. X-ray taken October 7, 1952, revealing the enlarged cardiac silhouette.

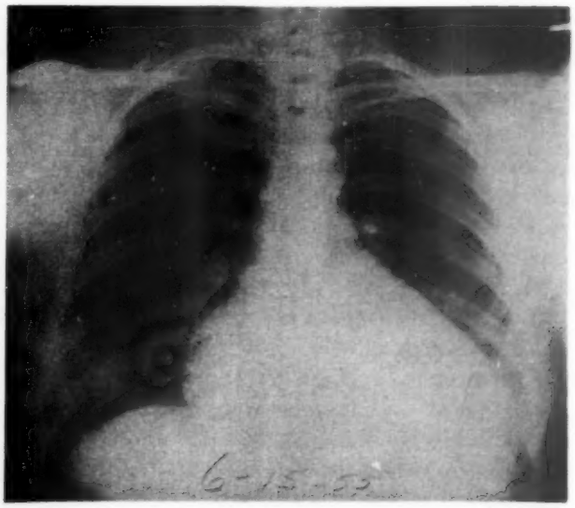


FIG. 2B. X-ray taken at initial examination in 1955. Note that the configuration is unchanged since the previous film in 1952.

sis. The hemoglobin was 14.6 gm. per 100 ml. and the sedimentation rate was 15 mm. per hour. The white blood cell count was 10,200 per cu. mm. with 78 per cent neutrophils, 18 per cent lymphocytes, 3 per cent monocytes and 1 per cent eosinophils. No. L.E. cells were seen on a peripheral blood preparation. The Kahn test was negative. Subsequent tuberculin, coccidioidin and histoplasmin skin tests were negative. A chest x-ray was reported as showing enlargement of the cardiac silhouette; the possibility of pericardial effusion was considered although myocardial dilatation could not be excluded.

The patient was admitted to Emory University Hospital on June 28, 1955, for further studies. At this time previous chest x-rays were reviewed, all of which, including the tuberculosis survey film taken seven years previously, revealed generalized enlargement of the cardiac silhouette. (Fig. 2.) An angiocardiogram was attempted, but there was extravasation of the dye into the arm muscles. An abdominal film taken one-half hour after the unsuccessful injection showed good renal opacification with normal outlines of the pelves and ureters. She was discharged on June 30, 1955, on no medications.

She was readmitted on August 16, 1955, and in the interim there had been no change in symptoms. Digitalis had been administered briefly without improvement. No change in the heart size was detected. Angiocardiography was successfully performed using 50 ml. of 70 per cent diodrast.® No shunt or obstruction was seen. The distance from the edge of the opacified heart chamber to the edge of the cardiac border was 4 cm. on both sides, without enlargement of the chambers. (Fig. 3A.) The findings were thought to be indicative of pericardial effusion.

Pericardial paracentesis via the subxiphoid approach was performed on August 17, and 850 ml. of thin odorless milky fluid, which did not immediately

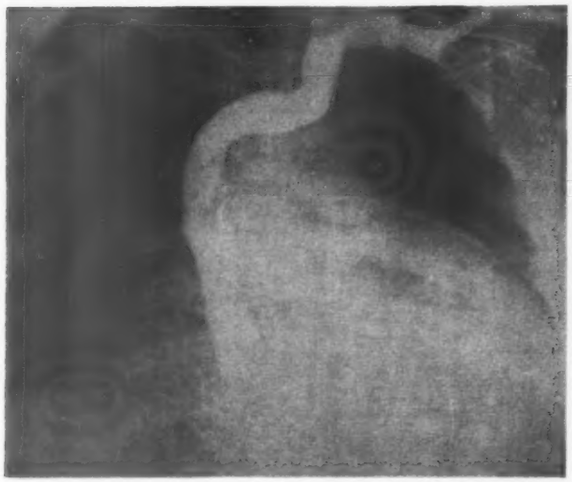


FIG. 3A. Angiocardiogram taken three seconds after injection. Note the distance between the dye column and the cardiac border. Later films showed a 4 cm. distance between the opacified heart chamber and the edge of the heart shadow.

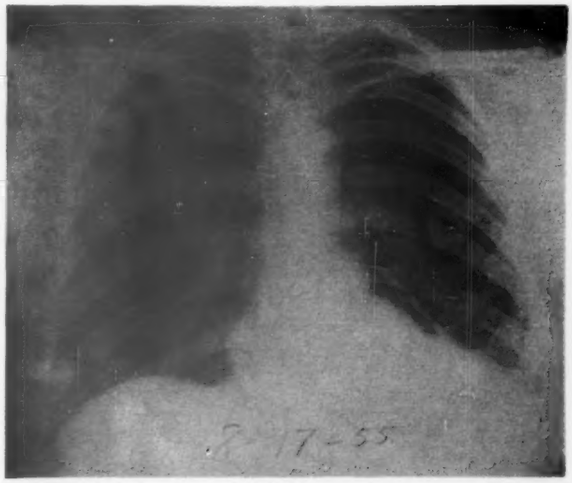


FIG. 3B. X-ray taken immediately after aspiration of 850 ml. of milky fluid and production of pneumopericardium with 50 ml. of air. The pericardium is smooth and measures 3 mm. in thickness. The heart shadow appears to be of normal size although there is some fluid remaining.

clot, were obtained. Approximately 50 ml. of air were instilled into the pericardial space and subsequent x-rays of the pneumopericardium revealed the pericardium to be smooth and 3 mm. in thickness. (Fig. 3B.) Although some fluid remained, the cardiac configuration appeared normal. The fluid contained rare red blood cells, 2,750 lymphocytes per cu. ml. The total protein was 5.4 gm. per 100 ml. and the cholesterol, 140 mg. per 100 ml. No cholesterol crystals were seen and no fat globules in a preparation stained with Sudan III. Routine cultures were negative as were cultures and guinea pig studies for tuberculosis. Papanicolaou stains for malignant cells were negative. The heart size diminished after the paracentesis and cardiac dullness extended only to the midclavicular line. A slight increase in voltage was seen on the electrocardiogram. A friction rub was heard over the precordium after the air instillation and the patient complained of slight substernal discomfort. She was discharged on August 19, and all adventitious heart sounds had disappeared by the fourth day following paracentesis.

The patient was next seen in the clinic on October 3, 1955, with complaints of occasional sighing respirations, easy fatigue, and feelings of depression. No great change in symptoms had been noted following pericardial paracentesis. The blood pressure was 150/90 mm. Hg without "paradox." General physical examination was unchanged and the weight remained stable. The cervical veins were flat and without unusual pulsations. Cardiac dullness again extended to the mid-clavicular line. Fluoroscopic examination revealed reaccumulation of the fluid, but cardiac pulsations were good in spite of the effusion. A low grade tuberculous infection was thought to be the underlying process and a therapeutic trial of streptomycin, 1.0 gm. intramuscularly every third day, and isoniazid, 300 mg. by mouth daily, was instituted.

On October 26, 1955, she was readmitted with a two-day history of chills, fever and malaise. The vital signs were normal and there was no evidence of cardiac tamponade in spite of evidence of continued pericardial effusion and a "parodoxic" pulse difference of 15 mm. Hg. On October 27, pericardial paracentesis was again performed with the removal of 530 ml. of milky fluid, believed to be chylous. The fat content was 0.6 gm. per 100 ml.; the cholesterol, 115 mg. per 100 ml.; the phospholipid, 11.5 mg. per 100 ml. and the fatty acid, 89 mEq. per L. The specific gravity was 1.014 and the pH 7.0. The evening after aspiration the temperature rose to 103°F. but subsided without therapy. Blood cultures were negative. The patient was thought to have drug fever and all medications were stopped. She was discharged afebrile on October 29, 1955.

When seen on December 14, 1955, she had no complaints other than mild exertional dyspnea and occasional substernal aching.

On February 9, 1956, the patient was readmitted with complaints of heaviness in the chest and slight exertional dyspnea, but orthopnea and dependent edema were denied. The substernal heaviness was greatest in the supine position. Physical examination revealed the blood pressure to be 140/90 mm. Hg with a paradoxic pulse difference of 12 mm. Hg. The pulse was 92 and regular. Other vital signs were normal. No abnormal pulsations were seen in the cervical veins. There was no change in the heart size. Since fluid from previous aspirations was thought to be chyle, an attempt was made to show the presence of a

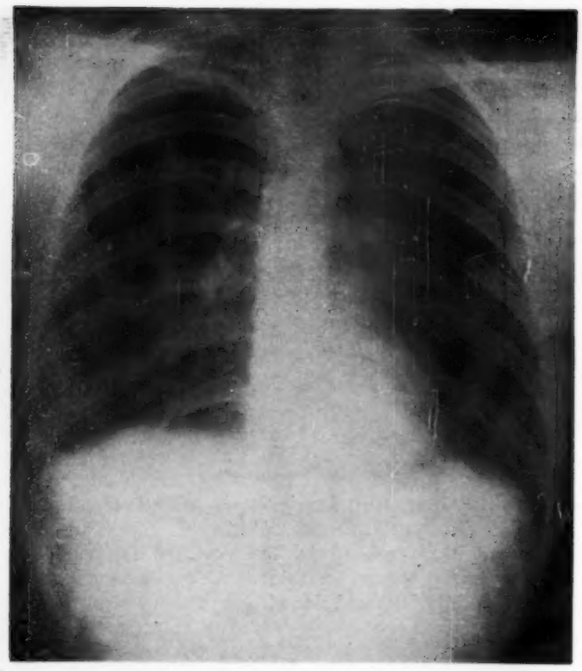


FIG. 4. X-ray taken two months after operation. Note the normal size of the cardiac silhouette.

communication with the thoracic duct. Pericardial paracentesis was performed with the removal of 250 ml. of milky fluid and immediately thereafter she was given 50 mg. of Sudan III in 20 ml. of warm olive oil by mouth. Twelve hours later a second pericardial paracentesis was performed and 465 ml. of milky fluid withdrawn. This fluid was extracted with an equal volume of ether and evaporated to dryness. The oily residue was orange in color, and on microscopic examination Sudan III–stained fat globules were seen. A control specimen subjected to the same procedure gave a light yellow residue. It was concluded that there was a communication between the thoracic duct and the pericardial cavity. Surgical exploration was advised.

On April 10, 1956, the patient was admitted to Emory University Hospital for surgery. Symptoms were unchanged. Her nutritional status was good, and no weight change had been noted. The blood pressure was 124/86 mm. Hg with an equivocal paradoxic difference. Slight cervical venous pulsations were seen in a 60 degree upright position. The apical impulse was not palpable and cardiac dullness extended to the mid-axillary line. The remainder of the physical examination was normal. Chest x-ray showed a globular cardiac configuration compatible with pericardial effusion, unchanged since June 16, 1955. Routine laboratory studies were within the normal range, although the sedimentation rate was 32 mm. per hour. The venous pressure was 80 mm. saline solution and the circulation time, arm to tongue (decholin), was eleven seconds. The preoperative electrocardiogram was unchanged.

On April 13, 1956, one hour after a fatty meal, operation was performed by Drs. William Van Fleit and Osler Abbott. The chest was entered through the bed of the right seventh rib, and upon opening the pleura no fluid was seen, although the pericardium was filled with whitish fluid under a pressure of 5 mm. Hg. The thoracic duct was identified but attempts to cannulate it were unsuccessful. The pericardial sac was then opened and several hundred milliliters of chylous fluid were aspirated. (Similar fluid was seen extending from the thoracic duct). The posterior pericardium was carefully examined but no communication was apparent. The parietal pericardium was thin and pliable; the visceral pericardium was whitish and slightly thickened, but in no way constricting. Exploration of the superior mediastium disclosed no tumor mass. Further dissection in the posterior mediastinum about the thoracic duct revealed large lymphatic channels coursing across the anterior surface of the vertebral column to the left side. By entering the left chest through the posterior mediastinum a large lymphatic channel was identified. There was no apparent communication with the pericardium. The large channel on the left, all collaterals and the thoracic duct were ligated and excised. A 4 cm. by 4 cm. window of pericardium was removed from the right anterolateral region of the pericardium and the area was left wide open for drainage into the right pleural space. Thoracotomy tubes were placed in the right chest and the wound closed. During the operation 1,400 ml. of whole blood were administered. The patient tolerated the procedure well.

The postoperative course was relatively uneventful. Portable chest x-ray on the second postoperative day revealed small pleural effusions bilaterally, but there was a definite decrease in the heart size. Repeat films on the ninth and fifteenth postoperative days showed continued minimal bilateral pleural effusions. Postoperative electrocardiograms showed a slight increase in the voltage and a slight shift in the mean electrical axis. The patient was discharged on the sixteenth postoperative day.

Microscopic examination of the pericardial sections revealed slight thickening of the pericardial tissue and a diffuse infiltration of polymorphonuclear cells in the dense fibrous tissue of the pericardium. There were no cholesterol deposits or foreign body giant cells. Sections of the thoracic duct showed some thickening and hyalinization of the wall, but no other significant findings.

Since the operation the patient has noted no awareness of substernal heaviness in the supine position and exercise tolerance has increased. The cardiac silhouette has returned to normal size and there has been no reaccumulation of the pericardial or pleural effusion (Fig. 4). Electrocardiograms continue to show a slight increase in voltage (Fig. 5) compared to preoperative tracings.

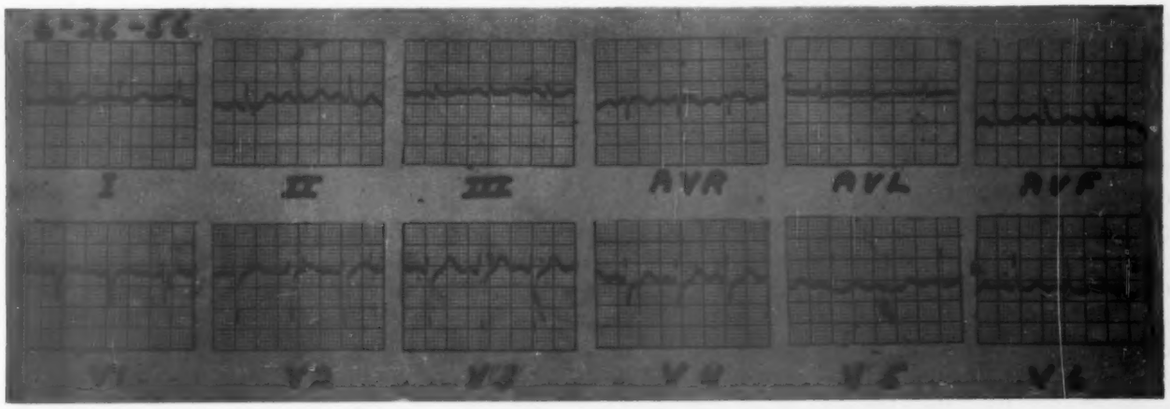


FIG. 5. Postoperative electrocardiogram showing increase in voltage. (1 cm. = 1 mv.).

COMMENTS

The subject of non-traumatic chylothorax and chylopericardium has been presented previously by Yater [*1*]. The most common etiology is primary or metastatic malignant disease resulting in obstruction to the duct and/or its radicles by implants within the lumen, or by pressure on the duct from without. There are included retroperitoneal and posterior mediastinal lymphoblastomas and abdominal carcinomas. Tuberculosis causing obstruction of the thoracic duct is next in importance and appears to be most common in children in whom pressure by lymph nodes or intrinsic obstruction is the basis for leakage. Other causes listed by Yater include lymphangiectasis, localized thrombosis of the subclavian vein in the region of the junction with the thoracic duct, filarial obstruction, thrombosis of the superior vena caval system, polyserositis, aneurysmal erosion and cirrhosis. The etiology in the only previously reported case of isolated or primary chylopericardium was a lymphangioma [*4*], and in the present case, unknown. Rupture of the thoracic duct and subsequent chylothorax has been reported following hyperextension of the spine [*6*], but it is hardly plausible that chylopericardium resulting from trauma twenty-three years previously could have produced such a paucity of symptoms. Thus the etiology of the chylous effusion remains undetermined. In spite of the enlarged cardiac silhouette presumably due to the chylous effusion present for at least eight years, the patient experienced only minimal symptoms. Except for pulsating neck veins on one occasion and at other times a slight increase in paradoxic pulse, no signs of cardiac tamponade were demonstrated. This problem has been discussed recently by Williams and Soutter [*7*] who stressed the importance of two physical signs, pulsus paradoxus and pulsating neck veins in the upright position.

Effusion of chyle results in symptoms of two general types: those due to pressure and those due to loss of chyle. These symptoms are prominent in chylothorax and can be noted in chylopericardium if frequent aspirations are necessary to relieve symptoms of cardiac tamponade. In the present case, symptoms were minimal, suggesting that the leakage was small, incomplete, or present over a long period of time with resultant distension of the pericardium due to the constant pressure. There was no external loss of chyle and therefore absence of weight loss and signs of malnutrition.

The appearance of a milky effusion containing fat should be considered presumptive evidence of its chylous nature. In both the case of Groves and Effler [*4*] and in the present one, this fact was not appreciated at the initial aspiration. The nature of chyle with its fat content makes the use of Sudan III of particular diagnostic importance. Sudan III does not stain neutral fat globules, which often constitute from 70 to 87 per cent of the total lipids of the chyle of experimental animals [*8*]. The method of oral administration of Sudan III in olive oil and subsequent extraction of chyle to demonstrate a communication between the pericardial sac and the thoracic duct was successful, but no communication could be demonstrated by inspection at operation. Unfortunately, the attempt to cannulate the thoracic duct and injection with an indicator to delineate the connection was unsuccessful also. Such a technic may be of aid in future cases. Klepser has advocated the administration

of olive oil and a lipophilic dye by duodenal tube several hours preceding operation to produce staining of the thoracic lymphatics [*9*].

Since chyle is bactericidal, it can be assumed that infections of chylous effusions will occur rarely and that the chance infection of the pericardial contents from aspiration or surgical procedures is highly improbable.

The relationship between cholesterol pericarditis and chylopericardium, if any, is unclear at the present time since only a few cases of each entity have been reported. The effusions in the former have been variously described as "golden yellow" [*11*], "holding in suspension an opalescent yellow material" [*12*] and "deep yellow" [*13*]. Characteristically, the fluid contains less cholesterol than the serum. No fat studies have been performed, although one fluid is reported as containing "occasional phagocytic cells filled with fat globules" [*12*]. Pericardial effusions containing quantities of cholesterol have been reported in myxedema, the levels ranging from 76 mg. per cent [*14*] to higher than the serum cholesterol [*15*]. Removal of opalescent or milky fluid from the pericardial sac should be followed by fat studies, including the administration of lipophilic dyes if indicated.

Ligation of the thoracic duct in the lower chest has been used in the treatment of chylothorax since Lampson, in 1948, first reported a successful case [*10*]. This method was used in the previously reported case of chylopericardium [*4*], together with removal of the lymphangioma and the creation of a pericardial window. Ligation of both thoracic lymphatic channels and the establishment of a pericardial window were used in the present case. An alternate method of treatment of chronic pericardial effusions consisting of the formation of a pericardial window and drainage into a pleural space or externally has been advocated [*5,7*]. This method may be of some value but the ultimate therapy appears to be combination of pericardial window formation and ligation of the thoracic duct low in the chest.

SUMMARY

The second case of a rare entity, isolated or primary chylopericardium, is reported. By the use of lipophilic dyes an anatomic communication between the thoracic duct and the pericardial cavity was demonstrated but at operation no direct communication was identified. The etiology of the chylous pericardial effusion in this case is unknown.

Ligation and excision of both major lymphatic channels and all collaterals low in the thorax together with the establishment of a pericardial window for drainage resulted in an apparent cure.

REFERENCES

1. Yater, W. M. Non-traumatic chylothorax and chylopericardium: review and report of case due to carcinomatous thromboangiitis obliterans of thoracic duct and upper great veins. *Ann. Int. Med.*, 9: 600, 1935.
2. Andrews, C. F. Traumatic intrathoracic rupture of thoracic duct with chylothorax. *Nebraska M. J.*, 14: 26, 1929.
3. Scannell, J. G. Discussion of Meade, R. H., Jr., Head, J. R. and Moen, C. W. The management of chylothorax. *J. Thoracic Surg.*, 19: 709, 1950.
4. Groves, L. K. and Effler, D. B. Primary chylopericardium. *New England J. Med.*, 250: 520, 1954.
5. Proudfit, W. L. and Effler, D. B. Diagnosis and treatment of cardiac pericarditis by pericardial biopsy. *J. A. M. A.*, 161: 188, 1956.
6. Shackelford, R. T. and Fisher, A. M. Traumatic chylothorax. *South. M. J.*, 31: 766, 1938.
7. Williams, C. and Soutter, L. Pericardial tamponade. *Arch. Int. Med.*, 94: 571, 1954.
8. Robinson, C. S., Cunningham, R. S., Blalock, A., Gray, M. E. and Rogers, B. C. Chylous effusions produced by experimental ligation of the superior vena cava. *Arch. Path.*, 24: 303, 1937.
9. Klepser, R. G. Discussion of Meade et al. [*3*].
10. Lampson, R. S. Traumatic chylothorax: a review of the literature and report of a case treated by mediastinal ligation of the thoracic duct. *J. Thoracic Surg.*, 17: 778, 1948.
11. Merrill, A. J. Cholesterol pericarditis. *Am. Heart J.*, 16: 505, 1938.
12. Ada, A. E. W., Jones, O. R. and Sheeran, A. D. Cholesterol pericarditis. *J. Thoracic Surg.*, 20: 28, 1950.
13. Creech, O., Jr., Hicks, W. M., Jr., Snyder, H. B. and Erickson, E. E. Cholesterol pericarditis. *Circulation*, 12: 193, 1955.
14. Harrell, G. T. and Johnston, C. Pericardial effusion in myxedema. *Am. Heart J.*, 25: 505, 1943.
15. Bullrinch, R. H., Repetto, R. and Ferrari, J. A. Myxedematous effusion. Chronic cardiac tamponade. *Prensa méd. argent.*, 31: 2423, 1944. Cited by Creech et al. [*13*].

Progesterone-Induced Porphyria*

Case Report

EDITHE J. LEVIT, M.D., JOHN H. NODINE, M.D. *and* WILLIAM H. PERLOFF, M.D.
Philadelphia, Pennsylvania

WATSON defines porphyria as a constitutional fault or inborn error in porphyrin metabolism.[1] The acute type occurs intermittently, with an incidence two to three times greater in women than in men. The disorder first becomes manifest during the second to fifth decades. It is characterized by the intermittent urinary excretion of large quantities of uroporphyrin and coproporphyrin with a resultant dark brown or Port wine color, acute colicky lower abdominal pain and various neurologic manifestations.[2] The initial attack may be noted either at the menarche or with a subsequent menstrual period.[3–7] It may also be associated with amenorrhea or menstrual irregularities,[5,8–13] and a tendency towards exacerbation at the time of the period has been reported repeatedly.[6,14–16] Severe porphyria following hysterectomy and bilateral salpingo-oophorectomy has been observed.[17–20] One report notes the occurrence of this metabolic disorder following deep x-ray therapy to the ovaries.[9]

The relationship of this interesting disease entity to pregnancy is somewhat uncertain. Whereas some investigators have observed the first attack following delivery,[5,9] others have reported it during pregnancy,[17,21–25] and still others believe its course to be uninfluenced by pregnancy in any way.[26–28]

It would appear likely from the foregoing that an endocrine factor is involved either as a causal or precipitating agent of acute porphyria in certain cases encountered in women. In view of this possible relationship, the following case report of a young woman with acute intermittent porphyria may be of interest. She was studied by the Endocrine Department at The Philadelphia General Hospital over a four-year period, with particular reference to her menstrual function.

CASE REPORT

P. D., a twenty-seven year old Negro woman, was seen in the Endocrine Clinic in August, 1952. She had been in good health until the age of twenty-three, when her menses first became irregular, occurring every two to three months. During the summer of 1951 she had an attack of what was later diagnosed as acute porphyria and was admitted to another hospital for treatment. She was readmitted to that hospital several months later with a similar episode, and then remained fairly well until July, 1952, when she suffered a third attack which lasted one week. This was followed by a menstrual period. Physical examination, including pelvic, was unrevealing. Except for serum serologic examinations positive for syphilis, Watson-Schwartz tests positive for porphobilinogen, all laboratory examinations including basal metabolic rate, cholesterol, twenty-four-hour thyroid I-131 uptake, urinary estrogens, gonadotropins, 17-ketosteroids and corticoids were negative. Her last menstrual period had occurred six weeks prior to this visit and two 50 mg. injections of progesterone (proluton®) were given intramuscularly forty-eight hours apart. A menstrual period ensued four days later, associated with an episode similar to the three preceding ones.

During the next two years (October, 1952 to October, 1954) the patient had a total of eight attacks, three of which followed progesterone-induced menstrual flow, four of which preceded spontaneous menstrual periods (which were ovulatory as evidenced by basal body temperature changes) and only one of which occurred during a period of amenorrhea. She had three spontaneous anovulatory periods during this time with no accompanying porphyria. On one additional occasion progesterone was given causing a menstrual period but without an attack of porphyria. 1 cc. doses of 1:2000 prostigmin® given on three successive days produced neither a menstrual flow nor an attack of porphyria.

In an effort to suppress ovulation and endogenous progesterone production and precipitate estrogen withdrawal bleeding, the patient was given piperazine

* From the Endocrine Clinics, Philadelphia General Hospital, Philadelphia, Pennsylvania.

estrone sulfate, 9 mg. daily for four weeks, following which an anovulatory period without complication was noted. Several weeks later, however, she began to spot spontaneously and an acute attack of porphyria requiring hospitalization followed. She was treated with ACTH during the acute stage, with marked relief of pain, and was maintained on cortisone for an additional two weeks during which she had a normal spontaneous uncomplicated period.

Following this hospitalization she had two spontaneous periods, both of which were pain-free and anovulatory. Preceding a subsequent menstrual period accompanied by a severe attack of porphyria, an endometrial biopsy specimen revealed a secretory endometrium.

For the next six months the patient was given 2 cc. injections of a long acting estrogen (delestrogen®) at monthly intervals, and responded with withdrawal bleeding to each one. Dark urine accompanied only one bleeding episode but no pain was noted during this period.

COMMENTS

Of particular interest in this case is the frequency with which an attack of acute porphyria could be precipitated with either endogenously produced or exogenously administered progesterone.[29] In the first three years of this study twelve attacks were associated with one or the other of these factors, whereas spontaneous menstrual periods which were anovulatory and in which progesterone was not produced were unaccompanied by porphyric episodes. Similarly, bleeding episodes following exogenous estrogen administration to inhibit ovulation were not complicated by attacks of porphyria. Meyer,[30] in 1954, reported the occurrence of an acute attack of porphyria following the injection of 25 mg. of progesterone. Stephenson,[31] in 1952 noted a corpus luteum cyst of the ovary on exploratory laparotomy in a patient with an attack of acute porphyria.

Prostigmin, which was given in this case in an attempt to induce a menstrual period, has been used in the treatment of acute porphyria with some success.[3,32,33] The administration of ACTH and cortisone resulted in dramatic improvement. Reports in the literature, however, are at variance, some cases showing improvement,[7,8,21,30] others showing little or no response.[7,8,18,34]

SUMMARY

A young woman with acute intermittent porphyria was followed over a four-year period with particular reference to her menstrual function. Studies indicate that progesterone, either endogenous or exogenous, induced attacks of acute porphyria in this patient.

Acknowledgments. The authors are grateful to Schering Corporation for the generous supply of progesterone (proluton®), to Abbott Laboratories for piperazine estrone sulfate (sulestrex®) and to E. R. Squibb and Sons for delestrogen.

REFERENCES

1. Cecil, R. L. A Textbook of Medicine. Philadelphia, 1948. W. B. Saunders Co.
2. Harrison, T. R. Principles of Internal Medicine. Philadelphia, 1950. Blakiston Co.
3. Berg, M. Acute porphyria. *Arch. Int. Med.*, 76: 335, 1945.
4. Clark, E. E. and Lawrence, H. E. Acute porphyria. Report of a case. *J. Nerv. & Ment. Dis.*, 108: 502, 1948.
5. Correll, H. L., Peters, B. J. and Murphy, F. D. Porphyria. A report of 2 cases. *Urol. & Cutan. Rev.*, 46: 341, 1942.
6. Gibson, Q. H., Harrison, D. C. and Montgomery, D. A. D. Case of acute porphyria. *Brit. M. J.*, 1: 275, 1950.
7. Oltman, J. E. and Friedman, S. Acute porphyria. A case showing the ineffectiveness of ACTH. *New England J. Med.*, 244: 173, 1951.
8. Olson, O. C. and Stiles, M. H. Cortisone therapy in porphyria hepatica, acute intermittent type. A report of unfavorable results. *Ann. Int. Med.*, 41: 357, 1954.
9. Grubschmidt, H. A. Case of acute porphyria. *California Med.*, 72: 243, 1950.
10. Michaels, J. P. Acute idiopathic porphyria. Report of a case. *South. M. J.*, 42: 965, 1949.
11. Nesbitt, S. Acute porphyria. *J. A. M. A.*, 124: 286, 1944.
12. Peters, G. A. Acute porphyria. Report of 2 cases. *Ann. Int. Med.*, 30: 1237, 1949.
13. Wheeler, F. R. Acute porphyria. Report of 2 cases. *Stanford M. Bull.*, 10: 15, 1952.
14. Grossfeld, E. Acute porphyria with unusual features. *Brit. M. J.*, 1: 1240, 1951.
15. Macleod, J. G. and Grant, I. W. B. Intermittent acute porphyria. *Edinburgh M. J.*, 60: 232, 1953.
16. Schwarz, G. A. and Moulton, J. A. Porphyria: a clinical and neuropathologic report. *Arch. Int. Med.*, 92: 221, 1954.
17. Kehoe, E. L., Newman, S. and Chandler, B. F. Acute porphyria. A problem in medical diagnosis. *U. S. Armed Forces M. J.*, 4: 1119, 1953.
18. Markovitz, M. Acute intermittent porphyria. Report of 5 cases and review of the literature. *Ann. Int. Med.*, 41: 1170, 1954.
19. Hare, E. H. Acute porphyria presenting with mental symptoms. *J. Ment. Sc.*, 99: 144, 1953.
20. Hart, F. D. and Gollard, P. Acute idiopathic porphyria presenting as progressive paresis of Landry type. *Brit. M. J.*, 1: 278, 1950.
21. Dean, G. Porphyria. *Brit. M. J.* 2: 1291, 1953.
22. Hetzel, B. S. Acute porphyria. 2 cases. *M. J. Australia*, 2: 600, 1949.
23. Kench, J. E., Ferguson, F. R. and Graveson, G. S.

Observations on 3 cases of acute porphyria. *Lancet*, 1: 1072, 1953.

24. Nieson, S. S. Pregnancy toxicosis and porphyria. *Nord. med.*, 44: 1178, 1950.
25. Reidenberg, L. and Farber, H. Acute intermittent porphyria complicated by pregnancy. *Am. J. Obst.*, 69: 879, 1955.
26. Coleman, R. R. Acute porphyria. *J. South Carolina M. A.*, 44: 117, 1948.
27. Freedman, A., Yeagley, J. D. and Brooks, J. B. Acute porphyria with improvement during and following pregnancy. *Ann. Int. Med.*, 36: 1111, 1952.
28. Hirson, C. Prognosis of acute porphyria. *Brit. M. J.*, 1: 1372, 1953.
29. Perloff, W. H., Frankel, D. and Bayne, G. M. Diagnostic and therapeutic uses of progesterone in clinical practice. *M. Times*, 81: 596, 1953.
30. Meyer, H., Nadler, S. B. and Bloch, T. Acute porphyria and pregnancy. *Obst. Gynec.*, 3: 214, 1954.
31. Stephenson, D. S. and Dominguez, A. Acute porphyria. 2 cases of idiopathic type. *Bol. Asoc. med. Puerto Rico.*, 44: 437, 1952.
32. Gillbespy, R. O. and Smith, S. G. Porphyria treated with neostigmine. *Lancet*, 266: 908, 1954.
33. Veflingstad, H. Prostigmine in porphyria. *Nord. med.*, 42: 1432, 1949.
34. Lewis, B. S. A case of acute porphyria. *J. Roy. Nav. M. Serv.*, 38: 146, 1952.

Chronic Disseminated Histiocytosis X (Schüller-Christian Disease) with Pulmonary Involvement and Impairment of Alveolar-Capillary Diffusion*

ATTILIO D. RENZETTI, JR., M.D., GERARD EASTMAN, M.D. *and* J. HOWLAND AUCHINCLOSS, JR., M.D.

Syracuse, New York

JAFFE and Lichtenstein [*1*] believe that the clinical conditions designated eosinophilic granuloma of bone, Letterer-Siwe disease and Schüller-Christian disease are different expressions of the same distinctive histiocytosis (granulomatosis) of unknown etiology, and Lichtenstein [*2*] has suggested that the name "Histiocytosis X" be used for all of them. According to this concept, the clinical differences among these three syndromes result from differences in the localization, number and extent of the individual lesions and from their differing rates of progression. Thus eosinophilic granuloma of bone represents a localized form of histiocytosis X. Although more than one bony lesion may be present, the response to curettement or X-ray therapy is good and the prognosis is generally excellent. However, visceral involvement by the same pathologic process may develop later than the bone disease and thereby indicate the disseminated form of the illness [*2*]. In its disseminated form histiocytosis X may be acute (Letterer-Siwe disease) or chronic (Schüller-Christian). The acute disease occurs almost exclusively in infancy and early childhood and is characterized by hepatosplenomegaly, generalized lymphadenopathy, anemia, fever and usually a rapidly fatal course. The chronic disease occurs in children and young adults and in its classic form presents the triad of bone defects, exophthalmos and diabetes insipidus. However, the clinical picture is frequently not classic but dominated by involvement of other areas of the body so that the patient may present with swelling of the gums, cyst-like swelling of the scalp, xanthoma disseminata or other cutaneous manifestations, or respiratory insufficiency. The course and prognosis in each case is largely determined by the extent of infiltration of such organs as the lungs, pituitary gland or bone marrow.

Rowland [*8*] in 1928 described a case of Schüller-Christian disease in which diffuse bilateral pulmonary infiltration was observed by x-ray and extensive pulmonary fibrosis was present at autopsy. In reviewing the literature up to that time he found three other instances of well documented lung involvement. Such involvement has since been described many times and in some instances, especially in the English literature, the term "honeycomb lungs" has been applied to it [*9*].

There have been numerous reports of generalized pulmonary infiltration by x-ray in patients with the skeletal lesions of eosinophilic granuloma. Recently biopsy of the lung in some of these patients [*3,4*] has revealed the same histologic lesion as that of the bone. According to Lichtenstein [*2*], these are also cases of Schüller-Christian disease or, as he would prefer to call them, histiocytosis X. In addition, there is now evidence that histiocytosis may be present in the lung in the absence of skeletal defects [*5,6*] and that pulmonary involvement may precede the appearance of bony lesions by as long as four years [*7*].

Two patients with eosinophilic granuloma of

* From the Chest Disease Division and Cardio-Pulmonary Laboratory of the Veterans Administration Hospital, and the Department of Medicine, State University of New York, College of Medicine, Syracuse, New York. This study was supported in part by a research grant (MR-7a-37) from the American Trudeau Society.

FIG. 1. Posteroanterior film of the chest taken in January, 1943.

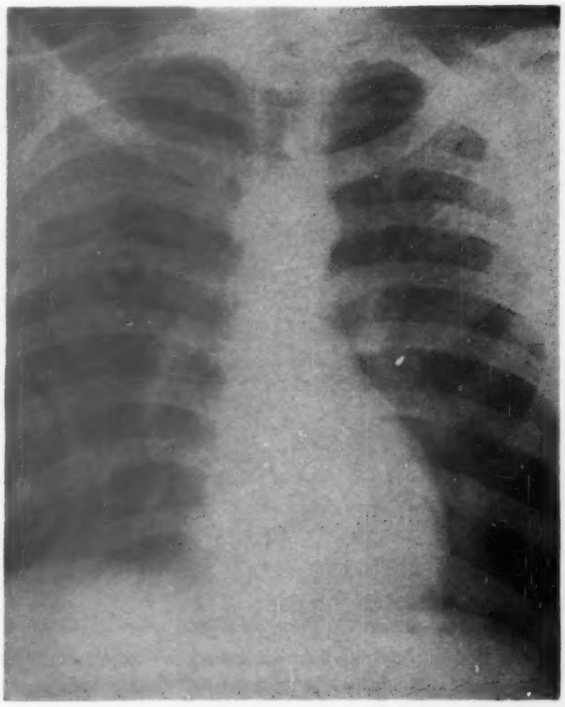


FIG. 2. Posteroanterior film of the chest taken in 1946.

bone and pulmonary infiltration which was demonstrated histologically to be granulomatous, have had pulmonary function studies. In one of these patients, Mazzitello [*6*] noted that except for a moderate increase in residual lung volume, the other lung volumes, as well as the maximum ventilatory capacity and arterial oxygen saturation at rest, were normal. No data are presented in the report of Childers et al. [*3*], but the authors state that hyperventilation and a decrease in the maximum ventilatory capacity were present.

The purpose of this paper is to report the case of a patient with chronic disseminated histiocytosis X whose pulmonary involvement was documented by x-ray from its onset and in whom studies of pulmonary function were carried out before and during therapy with adrenocortical steroids.

CASE REPORT

A thirty-two year old white man was admitted to the Syracuse Veterans Administration Hospital on October 22, 1955, complaining of shortness of breath for about one year. In January, 1943, upon entering military service, his chest x-ray was normal (Fig. 1) but in 1946, upon discharge from the service, his chest film (Fig. 2) showed a diffuse bilateral, finely nodular and linear infiltration with some coalescence in the upper lung fields. During a work-up in a tuberculosis sanatorium for this abnormality a barium study of the small intestine was performed. One of these x-rays (Fig. 3) showed an osteolytic lesion of the right ilium. This bony defect was not detected at that time and no diagnosis was made. However, about six months later, in early 1947, polydipsia and polyuria developed and the patient was admitted to the Syracuse University Hospital. There, in addition to the abnormalities of the lung and ilium revealed on x-ray, a bony defect was found in the left femur and a biopsy specimen of a right supraclavicular lymph node was interpreted as showing a non-lipid histiocytosis. His symptoms were attributed to diabetes insipidus and were well controlled by injections of pitressin.® Following discharge from the hospital he remained well. In 1948, a chest x-ray (Fig. 4) showed no significant change in his pulmonary disease but osteolytic lesions were then present in the skull (Fig. 5) and right humerus.

About one year before admission in October the patient noted shortness of breath upon exertion which became so severe as to require hospitalization at another hospital eight days before this admission. There he was observed to be cyanotic, without obtainable blood pressure or peripheral pulse and with a cardiac rate of 144 per minute. Following digitalization and diuresis he was transferred to the Syracuse Veterans Administration Hospital.

On physical examination he was tachypneic and moderately cyanotic. The temperature was 99.6°F., pulse 170, and blood pressure 82/60. Fine rales were

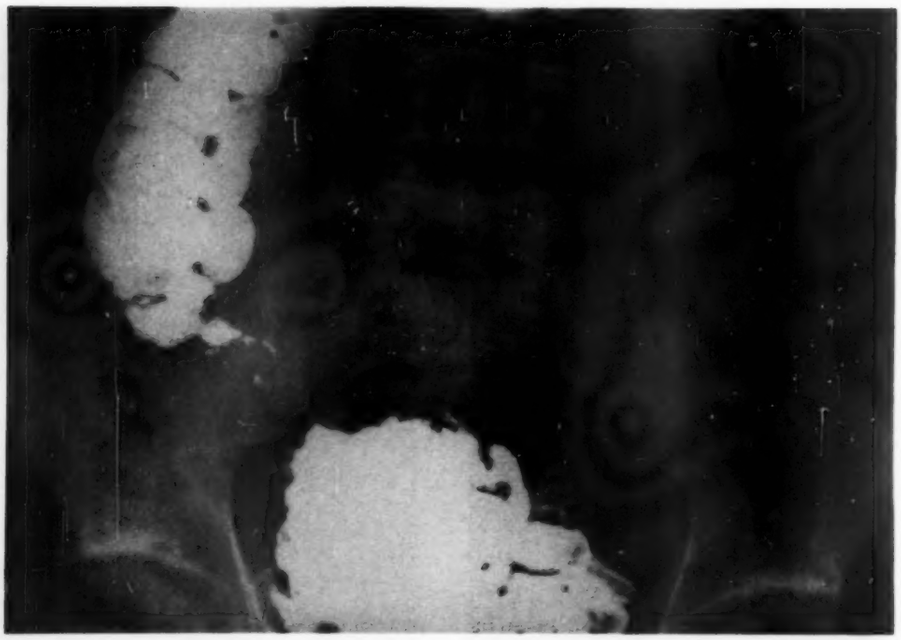


FIG. 3. Gastrointestinal x-ray taken in 1946. Note osteolytic lesions of ilium in left lower section of photograph.

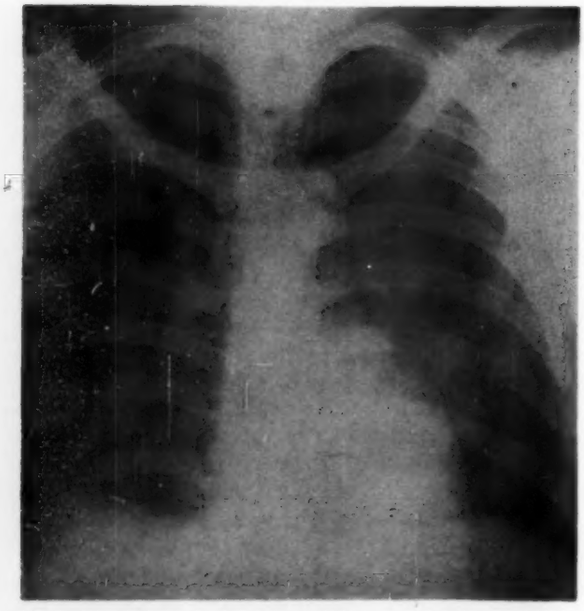


FIG. 4. Posteroanterior film of the chest taken in 1948.

present over the entire right lung posteriorly, at the left base posteriorly and the left upper anteriorly. A gallop rhythm was present over the right fourth intercostal space parasternally. The liver was palpable about five cm. below the right costal margin, and moderate clubbing of the fingers and toes was present.

Laboratory examination on admission revealed a hematocrit of 45 per cent, white blood cell count of 8,800 per cu. mm. with 75 per cent polymorphonuclear leukocytes, 17 per cent lymphocytes, 7 per cent monocytes and 1 per cent eosinophils. Urinalysis was normal except for 1+ albumin. The serum nonprotein nitrogen was 33 mg. per cent, CO_2 combining power 40 volumes per cent, sodium 137 mEq./L., potassium 5.2 mEq./L., chloride 92 mEq./L., thymol turbidity 2.6 units, alkaline phosphatase 3.0 Bodansky units. Electrocardiogram showed auricular tachycardia with the Wenckebach phenomenon. X-ray of the chest (Fig. 6) showed that the pulmonary infiltrations were more extensive and coalescent.

After treatment with oxygen and omission of digitalis there was a decrease in the cyanosis, tachypnea and tachycardia to such a degree that pulmonary function studies (Fig. 7) could be done on the fifth and sixth days after admission. Pitressin therapy was continued throughout hospitalization. On the seventh day, prednisone, 60 mg. per day, was started and within seventy-two hours rales could no longer be heard and oxygen was discontinued. Improvement was such that after a week of therapy the patient insisted that he be allowed to spend two days at home. After eight days of steroid therapy, moon facies had developed and prednisone dosage was reduced to 40 mg. per day. The occurrence of abdominal pain suggestive of peptic ulcer prompted further reduction in steroid dosage in step-wise fashion so that by the seventeenth day of therapy he was receiving 10 mg. prednisone per day. Pulmonary function studies were repeated on the seventeenth day of therapy. (Fig. 7.) Dyspnea recurred approximately ten days later and the patient required the administration of oxygen, digitalis, ACTH 40 units intramuscularly, and an

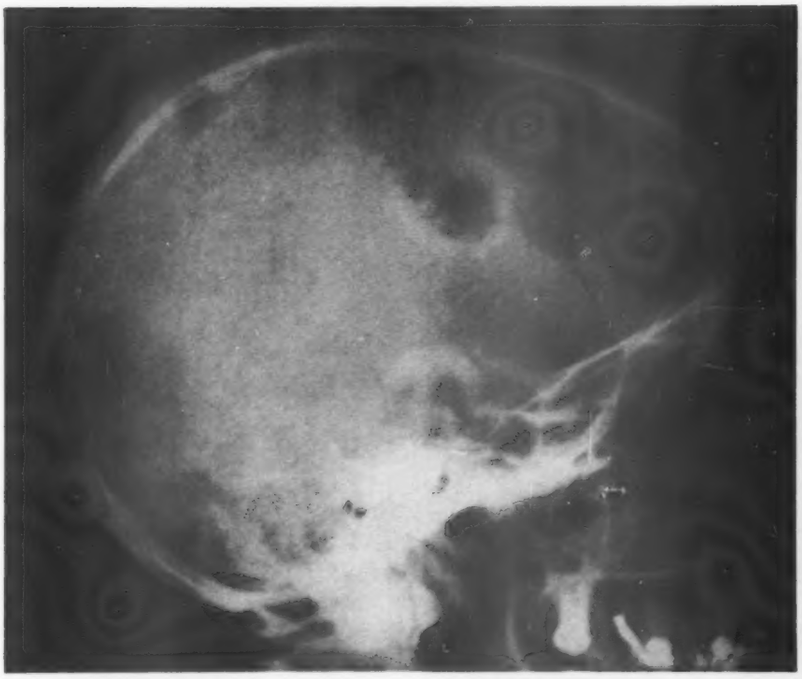


FIG. 5. X-ray of skull taken in 1948.

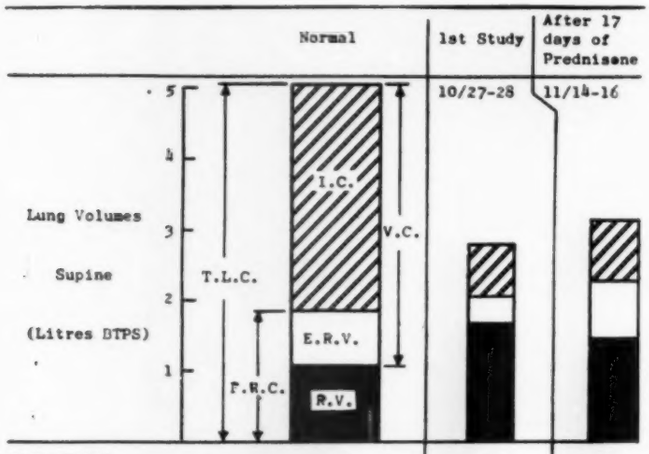
increase in prednisone dosage to 15 mg. per day thereafter. His condition seemed to improve for about five days but then chills, fever, rales over the right upper lung field anteriorly, increase in dyspnea, cyanosis and dependent edema developed. Following the administration of penicillin and streptomycin, there was an improvement in his condition again and he remained well until the forty-seventh hospital day when his temperature rose to 104°F. The patient became confused, and was found to have auricular tachycardia with varying degrees of A-V block. Shock developed and he died. A chest x-ray revealed no significant changes had taken place.

On autopsy the principal abnormal findings were in the respiratory, cardiovascular and hematopoietic systems. There were bilateral pleural fibrous adhesions and the lungs showed marked diffuse interstitial fibrosis and focal chronic interstitial pneumonitis. No etiologically specific changes were found for these findings. In addition there were multiple recent and organizing pulmonary emboli and hemorrhagic infarcts. The heart showed marked dilatation of the right ventricle and evidence of healed non-deforming mitral valvulitis and aortic valvulitis with early aortic stenosis. The valvular changes were thought by the pathologist to be on a rheumatic basis. In the vertebral bone marrow there was focal histiocytosis (Fig. 8) which was consistent with disseminated chronic histiocytosis. Concurrence in these pathologic diagnoses was obtained from the Armed Forces Institute of Pathology. Unfortunately, permission for examination of the head could not be obtained.

FIG. 6. Posteroanterior film of the chest taken in October, 1955.

METHODS

Measurements of lung volume, maximum ventilatory capacity, index of intrapulmonary mixing and



	Normal	1st Study	After 17 days of Prednisone
RV/TC X 100	20	63	43
Max. Vent. Cap. (L/min.)	118	38	68
Alv. N_2 after 7 min. O_2	< 2.5	1.5	1.6
Ventilation (L/min.)	5-7	21	22
O_2 Uptake (cc/min.)	200	280	286
Art. Pco_2 (mm. Hg)	36-40	46	28
Art. CO_2 Cont. (Vols. %)	46-50	65	41
Art. pH	7.36-7.42	7.49	7.51
Art. O_2 Sat. %	94-98	61	72
V_D^c/V_T^c X 100	< 30	72	64
$\dot{Q}va/\dot{Q}_T$ X 100	6% or less	34	22
D_{Lo_2} (cc/min./mm. Hg)	15	4	4
D_{Lco} (cc/min./mm. Hg)	10-20	3	4
$P_{Ao_2} - P_{Co_2}^e$ (mm. Hg)		59	48
$P_{Co_2}^e - P_{Ao_2}$ (mm. Hg)		10	14

Symbols

T.L.C. = total lung capacity
I.C. = inspiratory capacity
E.R. = expiratory reserve volume
V.C. = vital capacity
F.R.C. = functional residual capacity
R.V. = residual volume
V_D^c/V_T^c = ratio of physiological dead space to tidal volume where both are corrected for apparatus dead space
$\dot{Q}va/\dot{Q}_T$ = ratio of physiological venous admixture to total pulmonary blood flow
D_{Lo_2} = diffusing capacity of lung for oxygen
D_{Lco} = diffusing capacity of lung for carbon monoxide
$P_{Ao_2} - P_{Co_2}^e$ = oxygen tension difference between alveolar air and pulmonary capillary blood
$P_{Co_2}^e - P_{Ao_2}$ = oxygen tension difference between pulmonary capillary blood and arterial blood

Arterial blood and blood-gas tension values are those obtained during studies in which patient was breathing air at ambient oxygen tensions

FIG. 7. Results of pulmonary function studies before and after treatment with prednisone. Body surface area = 1.70 square meters. Arterial blood and blood-gas tension values are those obtained during studies in which the patient was breathing air at ambient oxygen tensions.

arterial oxygen saturation were made according to the methods described by Baldwin, Cournand and Richards [*10*]. The partial pressures of oxygen and carbon dioxide in arterial blood were obtained by the direct technic of Riley, Proemmel, and Franke [*11*]. Analysis of the ventilation-perfusion relationship and the diffusion characteristics of the lung at rest was made according to the methods described by Riley, Donald and Cournand [*12*]. In addition, the diffusing capacity of the lung for carbon monoxide was measured according to the method described by Filley [*13*], employing an infrared carbon monoxide analyzer.

RESULTS

The physiologic findings (Fig. 7) in this case are similar to those described by Austrian and his co-workers [*14*] in a group of patients with various granulomatous and fibrotic pulmonary diseases of both known and uncertain etiologies and by McClement and his associates [*15*] in patients who were in the acute phase of hematogenous pulmonary tuberculosis. The term "alveolar-capillary block" has been used to indicate the principal physiologic defect in these patients, namely, a reduction in the oxygen diffusing capacity of the lung. Although the maximum ventilatory capacity and arterial oxygen saturation at rest are usually normal or nearly normal in these patients, they may be considerably reduced in such an advanced stage of diffusional impairment as was manifested by the patient reported here. Further evidence of the severity of the physiologic disturbance in this patient is shown by the marked reduction of the diffusing capacity of the lung for oxygen and carbon monoxide and the extreme degree of hyperventilation. The increase in dead space-like ventilation and venous admixture-like perfusion must be at least in part an additional reflection of the severity of the barrier to diffusion since Donald and his associates [*16*] have pointed out that the diffusional block may be so great in some areas of the lung that carbon dioxide fails to diffuse completely. In such cases, the arbitrary division between distribution and diffusion abnormalities, on the basis of the absence

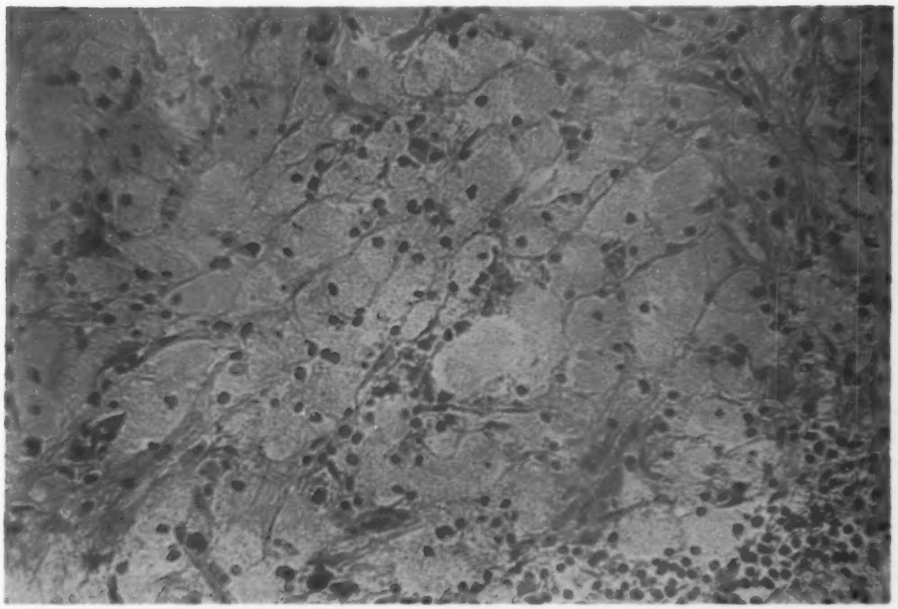


FIG. 8. Section of vertebral bone marrow obtained at autopsy. Large cells with foamy appearance of cytoplasm are histiocytes.

of a significant alveolar-capillary carbon dioxide gradient, is probably not valid.

Following treatment with prednisone, there was an improvement in lung volume, maximum ventilatory capacity and arterial blood gas content. (Fig. 7.) Since the diffusing capacity of the lung for oxygen and carbon monoxide was unchanged by treatment, the improvement in gas exchange must be attributed to the improvement of alveolar ventilation-perfusion relationships reflected in the reduction of the percentages of dead space and venous admixture. A similar response to steroid therapy has been noted previously [*17*] in patients with granulomatous lung disease and alveolar-capillary block. In view of the pathologic findings of focal interstitial pneumonitis in the patient reported here, it seems reasonable to assume that some resolution of an exudative or granulomatous process occurred during the course of prednisone therapy and thereby accounted for a better correlation between alveolar ventilation and capillary blood flow.

COMMENTS

The findings in this patient indicate that disseminated histiocytosis X should be considered among the diagnostic possibilities in cases of diffuse pulmonary disease of unknown etiology and, in addition to the usual studies, scalenus node biopsy and an x-ray survey of the entire skeleton should be done in such cases.

The need for early recognition and therapy of this disease is emphasized by the course of this man's illness. Since pitressin adequately controlled the symptoms resulting from pituitary involvement, it is possible that therapy directed at the granulomatous process may have avoided or delayed the fatal result of his pulmonary involvement. The pulmonary lesions reported as eosinophilic granuloma have been observed to clear spontaneously [*18*] or after treatment with radiation [*3*] or ACTH or cortisone [*6,18*]. However, the lung infiltration has also been noted to remain and progress despite the use of radiation [*4*] or steroid therapy [*7*]. It is of interest that when such lesions have been demonstrated by lung biopsy to be granulomatous rather than fibrotic, they have responded to either form of therapy [*3,6,18*]. In addition, the patient presented by Childers et al. demonstrated a return of pulmonary function to normal one year after radiation therapy had been administered. These findings suggest that in a manner similar to that observed in Boeck's sarcoid [*19*], both the physiologic defect and the parenchymal infiltration may be reversible when the pathologic process is cellular. Since severe pulmonary functional impairment leading to death may result when fibrosis occurs, it would seem advisable to treat the pulmonary lesions of this disease when such lesions progress or show no tendency to regress during observation.

CONCLUSIONS

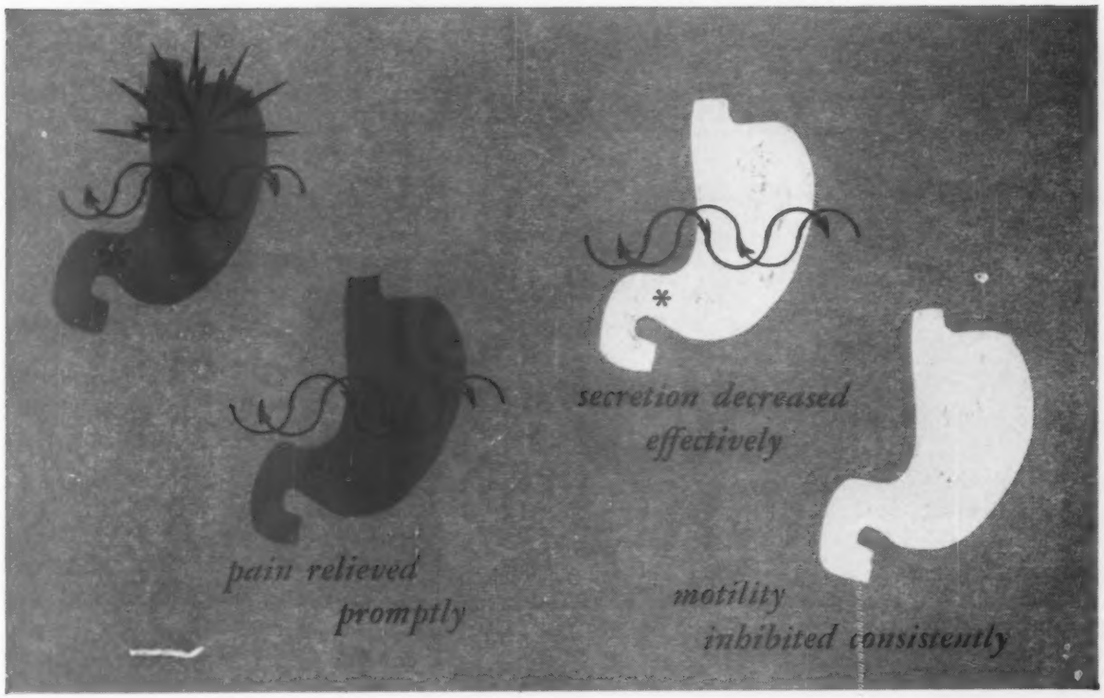
The pulmonary involvement by chronic disseminated histiocytosis X may result in extensive interstitial fibrosis of the lung and thereby give rise to the physiologic defect of alveolar-capillary block. Since death in this disease is frequently due to its effects on the lung, an effort should be made to determine the need for treatment with steroids or radiation in the earlier cellular phase of the process. Since early recognition of this disease may have important therapeutic implications, it is suggested that an x-ray survey of the entire skeleton be made in all young adults and children who have diffuse bilateral pulmonary disease of unknown etiology.

REFERENCES

1. Jaffe, H. L. and Lichtenstein, L. Eosinophilic granuloma of bone: a condition affecting one, several or many bones, but apparently limited to the skeleton, and representing the mildest clinical expression of the peculiar inflammatory histiocytosis also underlying Letterer-Siwe disease and Schüller-Christian disease. *Arch. Path.*, 37: 99, 1944.
2. Lichtenstein, L. Histiocytosis X. *Arch. Path.*, 56: 84, 1953.
3. Childers, J. H., Middleton, J. W. and Schneider, M. Eosinophilic granuloma of femur and lungs. *Ann. Int. Med.*, 42: 1297, 1955.
4. May, I. A., Garfinkle, J. M. and Dugan, D. J. Eosinophilic granuloma of lung: report of three cases. *Ann. Int. Med.*, 40: 549, 1954.
5. Farinacci, C. J., Jeffrey, H. C. and Lackey, R. W. Eosinophilic granuloma of the lung: report of 2 cases. *U. S. Armed Forces M. J.*, 2: 1085, 1951.
6. Mazzitello, W. F. Eosinophilic granuloma of the lung. *New England J. Med.*, 250: 804, 1954.
7. Bland, W. H., Levy, M. S. and Bassett, S. H. A case of Hand-Schüller-Christian syndrome treated with cortisone. *Ann. Int. Med.*, 35: 927, 1951.
8. Rowland, R. S. Xanthomatosis and the reticuloendothelial system. *Arch. Int. Med.*, 42: 611, 1928.
9. Oswald, N. and Parkinson, T. Honeycomb lungs. *Quart. J. Med.*, 18: 1, 1949.
10. Baldwin, E. deF., Cournand, A. and Richards, D. W., Jr. Pulmonary insufficiency. I. Physiological classification, clinical methods of analysis, standard values in normal subjects. *Medicine*, 27: 243, 1948.
11. Riley, R. L., Proemmel, D. D. and Franke, R. E. A direct method for determination of oxygen and carbon dioxide tensions in blood. *J. Biol. Chem.*, 161: 621, 1945.
12. Riley, R. L., Donald, K. W. and Cournand, A. Analysis of factors affecting the concentration of oxygen and carbon dioxide in the gas and blood of the lungs. II. Methods. *J. Appl. Physiol.*, 4:102, 1951.
13. Filley, G. F., MacIntosh, D. J. and Wright, G. W. Carbon monoxide uptake and pulmonary diffusing capacity in normal subjects at rest and during exercise. *J. Clin. Investigation*, 33: 530, 1954.
14. Austrian, R., McClement, J. H., Renzetti, A. D., Jr., Donald, K. W., Riley, R. L., and Cournand, A. Clinical and physiologic features of some types of pulmonary diseases with impairment of alveolar–capillary diffusion. *Am. J. Med.*, 11: 667, 1951.
15. McClement, J. H., Renzetti, A. D., Jr., Carroll, D., Himmelstein, A. and Cournand, A. Cardiopulmonary function in hematogenous pulmonary tuberculosis in patients receiving streptomycin therapy. *Am. Rev. Tuberc.*, 64: 583, 1951.
16. Donald, K. W., Renzetti, A., Riley, R. L. and Cournand, A. Analysis of factors affecting concentrations of oxygen and carbon dioxide in gas and blood of lungs. Results. *J. Appl. Physiol.*, 4: 497, 1952.
17. West, J. R., McClement, J. H., Carroll, D., Bliss, A. A., Kuschner, M., Richards, D. W., Jr. and Cournand, A. Effects of cortisone and ACTH in cases of chronic pulmonary disease with impairment of alveolar-capillary diffusion. *Am. J. Med.*, 10: 156, 1951.
18. Lackey, R. W., Leaver, F. Y. and Farinacci, C. J. Eosinophilic granuloma of the lung. *Radiology*, 59: 504, 1952.
19. McClement, J. H., Renzetti, A. D., Himmelstein, A. and Cournand, A. Cardiopulmonary function in the pulmonary form of Boeck's sarcoid and its modification by cortisone therapy. *Am. Rev. Tuberc.*, 67: 154, 1953.

CONFIRMED THERAPEUTIC UTILITY

Pro-Banthine®...
A Primary Drug in Peptic Ulcer

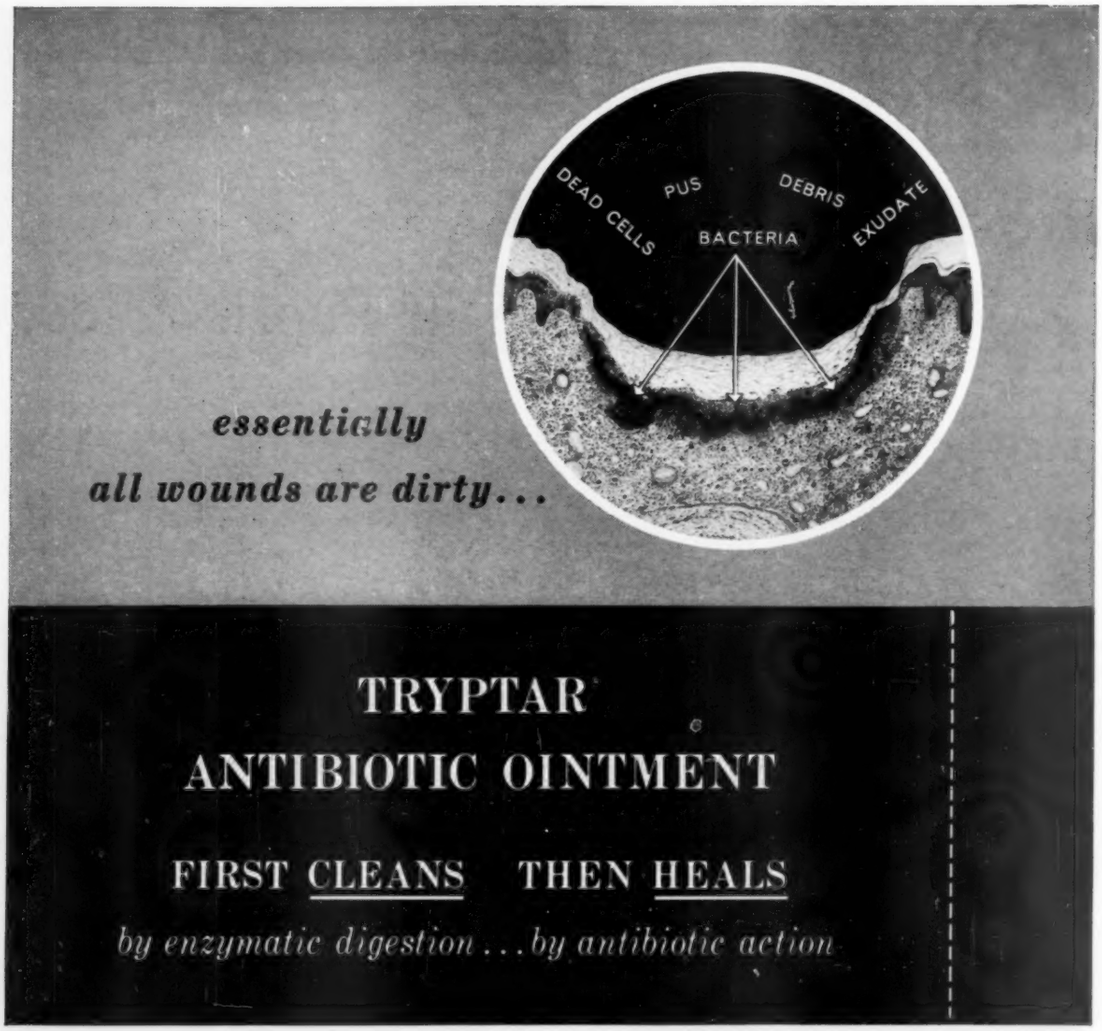


Among the many clinical indications for Pro-Banthine (brand of propantheline bromide), peptic ulcer is foremost. During treatment, Pro-Banthine has been shown repeatedly to be a singularly valuable agent when used in conjunction with diet, antacids, sedation and psychotherapy as required. Lichstein and his associates* report that Pro-Banthine "proved almost invariably effective in the relief of ulcer pain, in depressing gastric secretory volume and in inhibiting gastrointestinal motility. The incidence of side effects was minimal. . . ."

The therapeutic utility and effectiveness of Pro-Banthine in the treatment of peptic ulcer are repeatedly confirmed in the medical literature. Dosage: One tablet with each meal and two tablets at bedtime. G. D. Searle & Co., Chicago 80, Illinois, Research in the Service of Medicine.

*Lichstein, J.; Morehouse, M. G., and Osmon, K. L.: Pro-Banthine in the Treatment of Peptic Ulcer. A Clinical Evaluation with Gastric Secretory, Motility and Gastroscopic Studies. Report of 60 cases, Am. J. M. Sc. *232*:156 (Aug.) 1956.

SEARLE



Tryptar Antibiotic Ointment contains trypsin and chymotrypsin to clear away tissue debris, which then permits the two antibiotics, bacitracin and polymyxin, to exert full antibiotic power.

Tryptar Antibiotic Ointment can be easily applied to old, encrusted and infected wounds, as well as to fresh wounds. Its specially prepared water-soluble base facilitates removal of dirt, eschar and phagedenic membranes.

Tryptar Antibiotic Ointment may be applied as often as necessary, without fear of allergic reactions. There is no known contraindication to the use of Tryptar Antibiotic Ointment.

Each Gram of Tryptar Antibiotic Ointment Contains:

Trypsin (crystalline)	5,000 Armour Units
Chymotrypsin (crystalline)	5,000 Armour Units
Bacitracin U. S. P.	500 units
Polymyxin B Sulfate U.S.P.	5,000 units

in a specially prepared water-soluble ointment base.

THE ARMOUR LABORATORIES
A DIVISION OF ARMOUR AND COMPANY · KANKAKEE, ILLINOIS

in urinary tract
infections of pregnancy
delay is dangerous...

"Approximately one-half of the patients have some permanent damage to the urinary tract."[1]

FURADANTIN® *first...*

BRAND OF NITROFURANTOIN

FOR RAPID ERADICATION OF INFECTION

Specific for genitourinary tract infections • rapid bactericidal action against a wide range of gram-positive and gram-negative pathogens and organisms resistant to other agents • negligible development of bacterial resistance • excellent tolerance —nontoxic to kidneys, liver and blood-forming organs • safe for use in pregnancy[2,3]

AVERAGE FURADANTIN DOSAGE: 100 mg. q.i.d. with food or milk. Continue treatment for 3 days after urine becomes sterile.

SUPPLIED: Tablets, 50 and 100 mg. Oral Suspension (25 mg. per 5 cc. tsp.).

REFERENCES: 1. Rives, H. F.: Texas J. M. 52:224, 1956. 2. Diggs, E. S.; Prevost, E. C., and Valderas, J. G.: Am. J. Obst. 71:399, 1956. 3. MacLeod, P. F., et al.: Internat. Rec. Med. 169:561, 1956.

NITROFURANS

a new class of antimicrobials—neither antibiotics nor sulfonamides

EATON LABORATORIES NORWICH, NEW YORK

abnormal capillary permeability and fragility frequently occur in and are aggravated by...

- habitual and threatened abortion
- certain respiratory infections
- diabetic and other retinopathies
- gingival bleeding; epistaxis
- hypertension
- gastrointestinal bleeding

C.V.P. is a specific aid in the prevention and correction of capillary fault in such conditions

C.V.P.

Each C.V.P. capsule
or each 5 cc. of syrup
(approx. one teaspoonful) provides:
Citrus Bioflavonoid Compound . 100 mg.
Ascorbic Acid (vitamin C) . . . 100 mg.

capsules: bottles of 36, 100, 500, 1000.
syrup: bottles of 4 oz., 16 oz. and gallon.

new!

duo-C.V.P.

(double strength C.V.P.)

Each duo-C.V.P. capsule provides:
Citrus Bioflavonoid Compound . 200 mg.
Ascorbic Acid (vitamin C) . . . 200 mg.

capsules: bottles of 100, 500 and 1000.

C.V.P. helps diminish increased capillary permeability, fragility, and resultant bleeding by acting to maintain the integrity of the intercellular cement of capillary walls.

C.V.P. provides the many active bioflavonoid factors of the whole citrus bioflavonoid complex. C.V.P. is water-soluble and is thus readily absorbed and utilized, in contrast to purified hesperidin and rutin which are poorly soluble in water.

SAMPLES on request

u. s. vitamin corporation
(Arlington-Funk Labs., division)
250 E. 43rd St., New York 17, N. Y.

in convalescence

one of many indications for

Myadec®

high potency vitamin-mineral formula

"Generally, the more rapid and complete the nutritional rehabilitation, the shorter the convalescence."*

MYADEC Capsules are supplied in bottles of 30, 100, 250, and 1,000.

*Goodhart, H. S.: Vitamin Therapy Today, *M. Clin. North America* 40:1473, 1956.

PARKE, DAVIS & COMPANY • DETROIT 32, MICHIGAN

50119

66th YEAR OF PUBLICATION

The Leading Independent Surgical Journal

PRESENTS THE PAPERS OF . . .

The American Association for the Surgery of Trauma

•

The Pacific Coast Surgical Association

•

The American Society of Maxillofacial Surgeons

•

OTHER REGULAR FEATURES . . .

Modern Operative Technics
Practical Surgical Suggestions
New Instruments
Book Reviews

START YOUR SUBSCRIPTION WITH THIS ISSUE

The American Journal of Surgery
49 West 45th St., New York 36, N. Y.

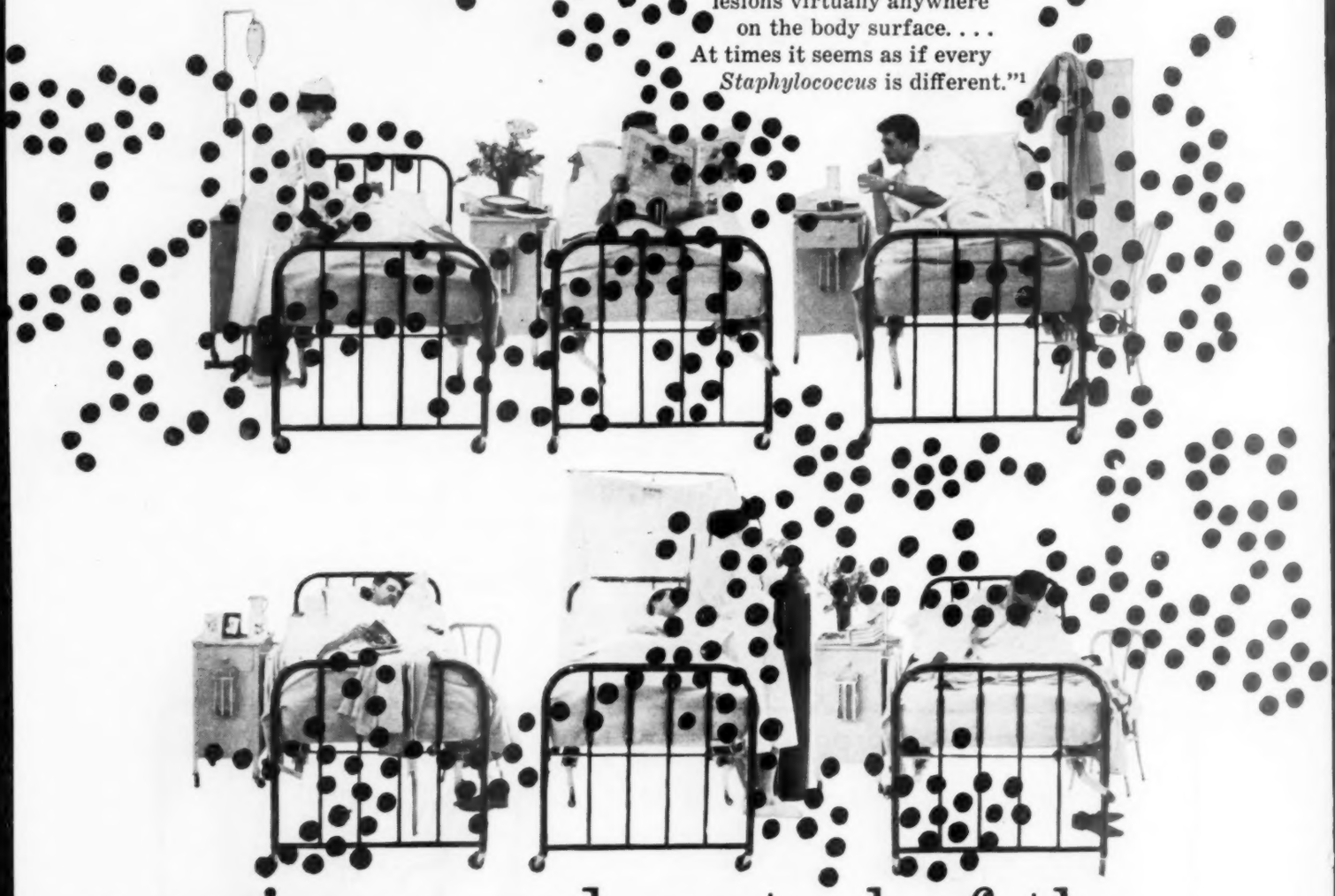
Please enter my subscription to The American Journal of Surgery. Yearly $15.00 U.S.A.—$16.00 Canada—$17.00 Foreign

Name .....

Address .....

City..... Zone..... State.....

The ubiquitous microbe
". . . capable of producing
lesions virtually anywhere
on the body surface. . . .
At times it seems as if every
Staphylococcus is different."[1]



now improved control of the ubiquitous Staph. with new

MATROMYCIN®

BRAND OF OLEANDOMYCIN

for the common bacterial infections that you treat with antibiotics other than broad spectrum . . . clinical success even in cases of antibiotic-resistant staphylococci

- no predictable cross resistance with penicillin, erythromycin, streptomycin, tetracycline, oxytetracycline and chlortetracycline
- resistance to Matromycin itself does not readily occur and emerges slowly and in adaptive fashion, as shown by experiments with various strains of *M. pyogenes* (clinical isolates)[2]
- outstandingly safe and well tolerated

Available in 250 mg. capsules, bottles of 16

references: 1. McDermott, W.: Ann. New York Acad. Sc. *65*:59 (Aug. 31) 1956. 2. Noyes, H. E.; Nagle, S. C., Jr.; Sanford, J. P., and Robbins, M. L.: Antibiotics & Chemother. *6*:450 (July) 1956.

Pfizer PFIZER LABORATORIES, Brooklyn 6, N.Y.
Division, Chas. Pfizer & Co., Inc.
World leader in antibiotic development and production

gentle

is the word

for Noludar

Mild, yet positive in action, Noludar 'Roche' is especially suited for the tense patient who needs to relax and remain clear-headed—or for the insomniac who wants a refreshing night's sleep without hangover. Not a barbiturate, not habit-forming. Tablets, 50 and 200 mg; elixir, 50 mg per teasp.

Noludar® brand of methyprylon
(3,3-diethyl-5-methyl-
2,4-piperidinedione)

ROCHE

Original Research in
Medicine and Chemistry

each dose is fresh
...for complete potency

FOLBESYN*

VITAMINS LEDERLE
B COMPLEX + C

Separate packaging of dry vitamins and diluent (mixed immediately before injection) assure controlled dosage. The folic acid solution is specially prepared to preserve full potency and to serve for quick solution of the dried vitamins. FOLBESYN may be conveniently added to standard intravenous solutions.

Dosage: 2 cc. daily.

Each 2 cc. dose contains:

Thiamine HCl (B_1)	10 mg.
Riboflavin (B_2)	10 mg.
Niacinamide	50 mg.
Pyridoxine HCl (B_6)	5 mg.
Sodium Pantothenate	10 mg.
Ascorbic Acid (C)	300 mg.
Folic Acid	3 mg.
Vitamin B_{12}	15 mcgm.

Lederle LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID COMPANY, PEARL RIVER, N. Y.
*REG. U. S. PAT. OFF.

Now, convenient B.I.D.

in more effective broad-spectrum

Clinically confirmed:
On a time-and-labor saving dosage schedule of 500 mg. (2 capsules) twice-a-day, Tetrex is as therapeutically effective and as free from adverse side effects as on 250 mg. (1 capsule) q.i.d.

Average adult dose: 1 Gm. per day in 2 doses of 500 mg. (2 capsules) each, or in 4 doses of 250 mg. (1 capsule) each.

Tet

TETRACYCLINE

—each capsule equivalent to 250 mg.

Bristol

For the Aged and Senile Patient

ORAL *Metrazol*

— to help the geriatric patient with early or advanced signs of mental confusion attain a more optimistic outlook on life, to be more cooperative and alert, often with improvement in appetite and sleep pattern.
Metrazol, a centrally acting stimulant, increases respiratory and circulatory efficiency without over-excitation or hypertensive effect.

Dose: 1½ to 3 grains, 1 or 2 teaspoonfuls Liquidum, or the tablets, every three or four hours.

Metrazol tablets, 1½ grs. (100 mg.) each. Metrazol Liquidum, a wine-like flavored 15 per cent alcoholic elixir containing 100 mg. Metrazol and 1 mg. thiamine HCl per teaspoonful.

Metrazol®, brand of pentylenetetrazol, a product of E. Bilhuber, Inc.

BILHUBER-KNOLL CORP. *distributor* ORANGE NEW JERSEY

dosage

antibiotic therapy with

rex™ CAPSULES

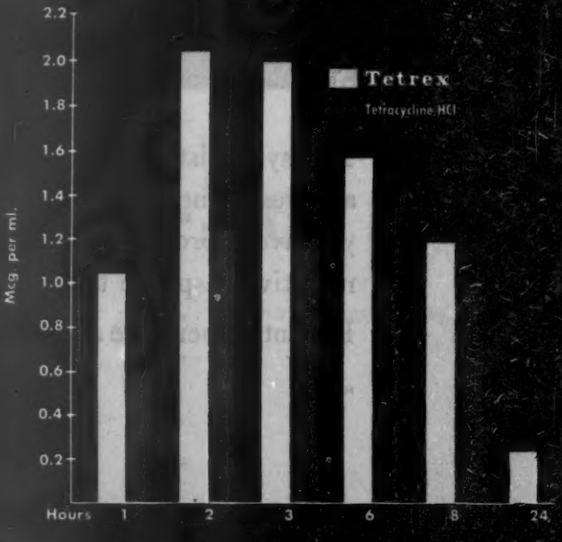
PHOSPHATE COMPLEX

tetracycline hydrochloride

Tetrex—the new Bristol tetracycline—facilitates earlier and more successful control of infection through faster and higher blood levels, practically double those of tetracycline HCl within 1 to 3 hours after administration of a single capsule. Yet Tetrex costs no more than the older tetracycline HCl.

Blood levels practically double after single capsule dose of Tetrex

— from 3 independent interim studies by P. A. Bunn, Sol Katz, and G. A. Cronk, on 188 patients.



The synergistic action of Nembutal® and reserpine in Nembu-Serpin helps you avoid prolonged waiting for a cumulative response to reserpine alone.

Patients experience a new sense of calm and well-being from the very first day of Nembu-Serpin treatment.

And fast-acting Nembu-Serpin makes lower reserpine dosages effective, reduces the incidence of side effects. Com-

701689

calmer days...more restful nights beginning first day of treatment

bines 30 mg. Nembutal Calcium and 0.25 mg. reserpine.

for milder cases/for maintenance therapy: Nembu-Serpin is also available in ½ strength, combining just 15 mg. Nembutal Calcium and 0.1 mg. reserpine in each Filmtab.

Abbott

FILMTAB® Nembu-Serpin®

®Filmtab—Film-sealed tablets

®Nembutal—Pentobarbital, Abbott

PSORIASIS

Proved Clinically Effective Oral Therapy—

maintenance regimen may keep patients lesion-free.

COMPLETE LITERATURE AND REPRINTS
UPON REQUEST. JUST SEND AN Rx BLANK.

LIPAN®

LIPAN Capsules contain: Specially prepared highly activated, desiccated and defatted *whole* Pancreas: Thiamin HCl, 1.5 mg. Vitamin D, 500 I.U.

Available: Bottles 180's, 500's.

Spirt & Co., Inc.
WATERBURY, CONN.

©Copyright 1956 Spirt & Co.

A "sense of well-being" is an added benefit in "Premarin" therapy

Every woman who suffers in the menopause deserves "Premarin," widely used natural, oral estrogen.

"Premarin" produces prompt symptomatic relief and a gratifying "sense of well-being." "Premarin" presents the complete equine estrogen-complex. Has no odor, imparts no odor.

"PREMARIN"®

Conjugated estrogens (equine)

in the menopause and the pre- and postmenopausal syndrome

5642 Ayerst **Ayerst Laboratories • New York, N. Y. • Montreal, Canada**

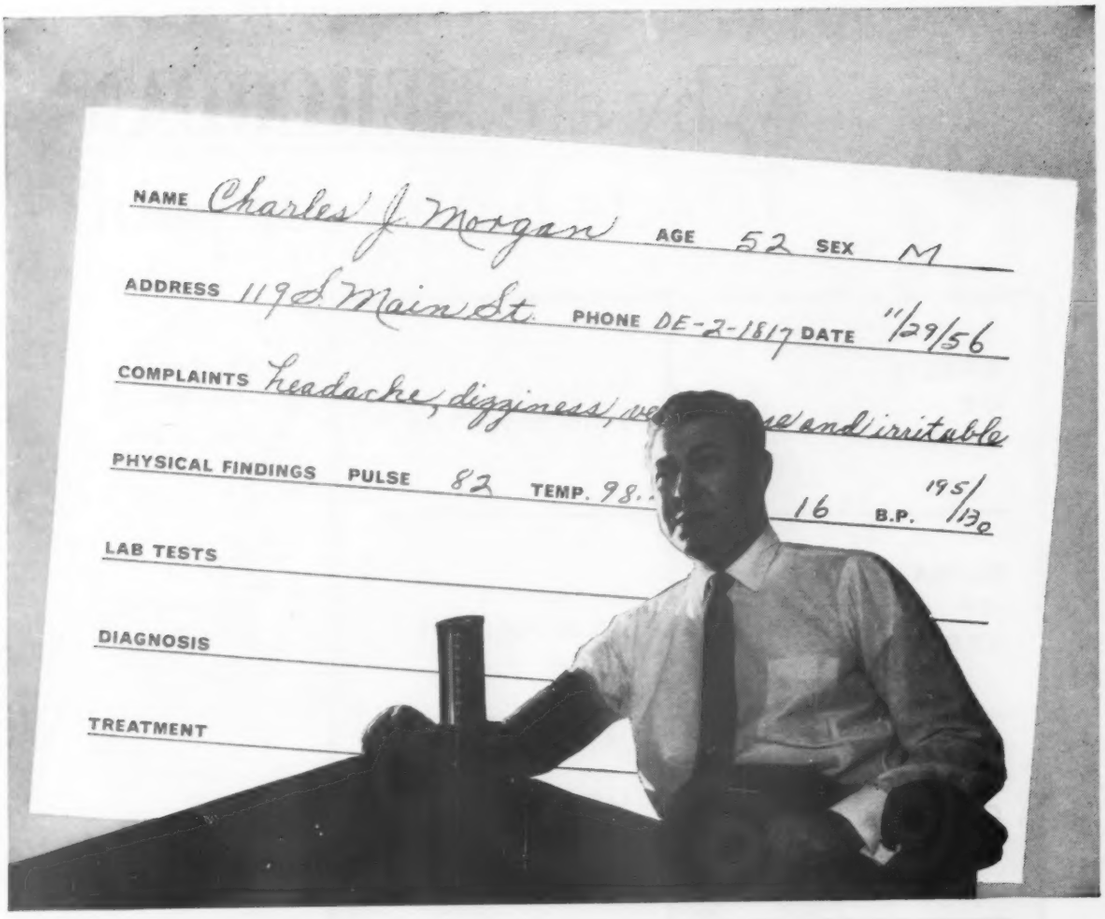
For round-the-clock therapy

With two doses a day

Lipo Gantrisin 'Roche'—a new, palatable liquid for antibacterial therapy—offers three significant features:

1. Only two doses a day needed in most cases
2. Adequate twelve-hour blood levels after a single dose
3. Same therapeutic advantages as Gantrisin 'Roche'

Lipo Gantrisin® Acetyl—brand of acetyl sulfisoxazole in vegetable oil emulsion


NAME Charles J. Morgan AGE 52 SEX M
ADDRESS 119 S Main St. PHONE DE-2-1817 DATE 11/29/56
COMPLAINTS headache, dizziness, we
and irritable
PHYSICAL FINDINGS PULSE 82 TEMP. 98.
16 B.P. 195/130
LAB TESTS
DIAGNOSIS
TREATMENT

Here is Effective Antihypertensive therapy WITH GREATER SAFETY

full effects with smaller doses

Alseroxylon and alkavervir combined are much more effective than either drug used alone.[1] The resulting additive if not synergistic action[2] provides full antihypertensive effects with relatively smaller doses[3] of each component drug and with fewer side actions.

Each scored tablet of Rauvera contains 1 mg. alseroxylon and 3 mg. alkavervir.

safe initial and routine therapy

Safety is a distinguishing feature of *Rauvera*, a potent antihypertensive agent with safeguards inherent in the purified mixed alkaloid fractions, alseroxylon and alkavervir. The risks of depression,[4] postural hypotension or a reduction of blood pressure to undesirable levels are virtually absent with *Rauvera*. Patients can be started routinely on *Rauvera*. Therapy can be continued over long periods of time.

1. LaBarbera, J.F.: Med.Rec. & Ann. *50*:242, 1956. 2. Ledbetter, P.V., and Morrow, E.J.: J. Am. Geriatrics Soc. *3*:172 (March) 1955. 3. Wilkins, R.W.: Am. J. Med. *17*:703 (Nov.) 1954. 4. Moyer, J.H.; Dennis, E., and Ford, R.: A.M.A. Arch. Int. Med. *96*:530, 1955.

RAUVERA®

SMITH-DORSEY • a division of The Wander Company • Lincoln, Nebraska

Why are **PERCODAN**® Tablets better for pain?

SPEED OF ACTION	WITHIN 5-15 MINUTES[1-3]	almost immediate relief of pain
DURATION OF EFFECT	6 HOURS AND LONGER[1-3]	sleep uninterrupted by pain
THOROUGHNESS OF PAIN RELIEF	USUALLY COMPLETE[1-5]	reliability of pain relief
INCIDENCE OF CONSTIPATION	RARE[1-3]	excellent for chronic and bedridden patients

Better than codeine plus APC[1-5]

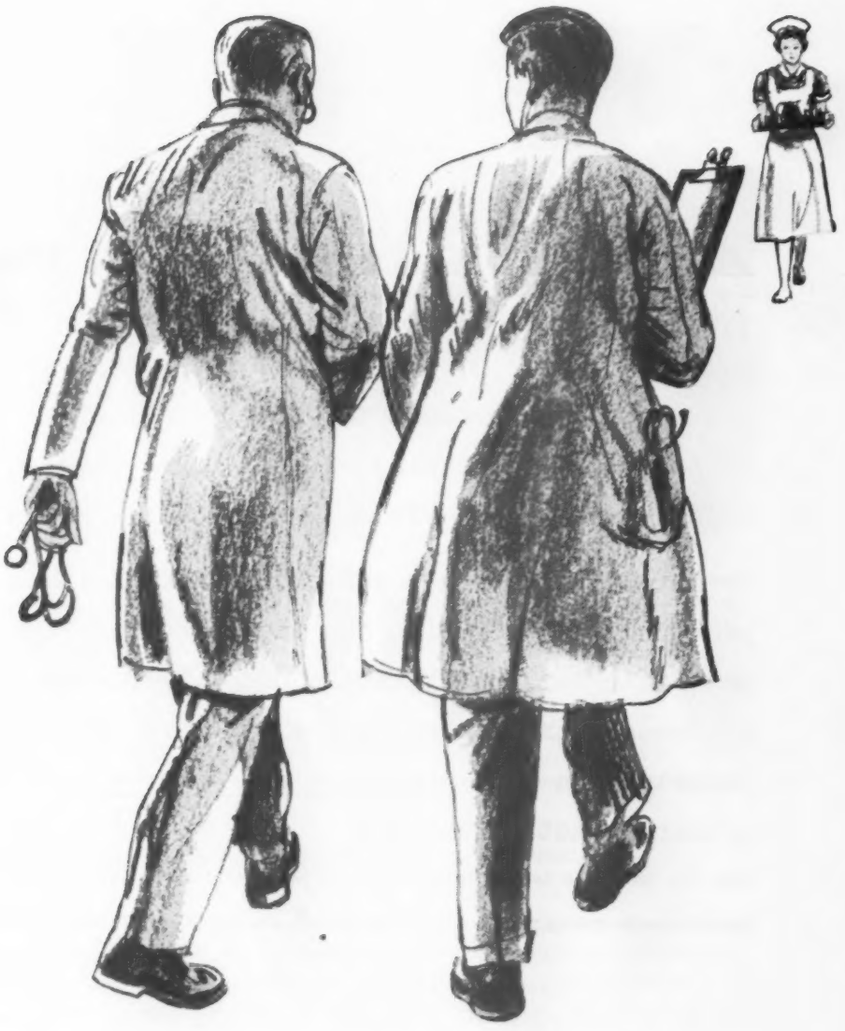
Average adult dose: 1 PERCODAN* Tablet every 6 hours.

Supplied: Scored, yellow oral tablets, containing salts of dihydrohydroxycodeinone and homatropine, plus APC. May be habit-forming. PERCODAN Tablets are available at all pharmacies.

References: 1. Piper, C. E., and Nicklas, F. W.: Indust. Med. 23:510, 1954. 2. Blank, P., and Boas, H.: Ann. West. Med. & Surg. 6:376, 1952. 3. Chasko, W. J.: J. District of Columbia Dent. Soc. 31:3, No. 5, 1956. 4. Cass, L. J., and Frederick, W. S.: M. Times 84:1318, 1956. 5. Bonica, J. J.: GP 10:35, No. 5, 1954.

Literature? write Endo® **ENDO LABORATORIES,** Richmond Hill 18, New York

*U.S. Pat. 2,628,185



" . . . Well, I always prescribe Rorer's Maalox. It's an excellent antacid, doesn't constipate and patients will take it indefinitely."

. . . . . .

MAALOX® suspension, bottles of 12 fluidounces *(sample on request)*; **tablets, bottles of 100.**

An efficient antacid suspension of magnesium-aluminum hydroxide gel; tablets, 0.4 Gm.

WILLIAM H. RORER, INC. 4865 Stenton Ave., Philadelphia 44, Pennsylvania

Against Pathogen & Pain

in urinary tract infections

Azo Gantrisin combines the single, soluble sulfonamide, Gantrisin, with a time-tested urinary analgesic - in a single tablet.

Prompt relief of pain and other discomfort is provided together with the wide-spectrum antibacterial effectiveness of Gantrisin which achieves both high urinary and plasma levels so important in both ascending and descending urinary tract infections.

Each Azo Gantrisin tablet contains 0.5 Gm Gantrisin 'Roche' plus 50 mg phenylazo-diamino-pyridine HCl. Gantrisin® - brand of sulfisoxazole

ROCHE | Original Research in Medicine and Chemistry

specifically for reduction of overweight

PRELUDIN®

(brand of phenmetrazine hydrochloride)

"...a highly effective and safe appetite suppressant..."[1]

Based on clinical reports, PRELUDIN produces more than twice the weight loss achieved by patients receiving a placebo.[2] It is singularly free of tendency to produce serious side actions, as well as stimulation.[1-3] PRELUDIN imparts a feeling of well-being that encourages the patient to cooperate willingly in treatment.[1-3]

The reduced incidence of side actions with PRELUDIN makes losing weight more comfortable for the average patient, facilitates treatment of the complicated case and frequently permits its use where other anorexiants are not tolerated.[3]

Recommended Dosage: One tablet two to three times daily one hour before meals. Occasionally smaller dosage suffices. On theoretical grounds, PRELUDIN should not be given to patients with severe hypertension, thyrotoxicosis or acute coronary disease.

(1) Holt, J. O. S., Jr.: Dallas Med. J. 42:497, 1956. (2) Gelvin, E. P.; McGavack, T. H., and Kenigsberg, S.: Am. J. Digest. Dis. 1:155, 1956. (3) Natenshon, A. L.: Am. Pract. & Digest Treat. 7:1456, 1956.

PRELUDIN® (brand of phenmetrazine hydrochloride). Scored, square, pink tablets of 25 mg. Under license from C. H. Boehringer Sohn, Ingelheim.

GEIGY Ardsley, New York

77857

Relieved of symptoms but still alert...
is the patient for whom you prescribe

CLISTIN®

Carbinoxamine Maleate

for "...effective control of allergic symptoms with little risk of sedation..."[1]

A "...potent antihistaminic drug with only weak sedative properties..."[2] Clistin is right

...for the patient who must remain wide awake and on the job in spite of his allergy

...for the very young allergy sufferer

...for ALL your allergy patients

CLISTIN dosage forms:
Tablets Clistin, 4 mg.
Tablets Clistin R-A (Repeat Action Tablets Clistin, 8 mg.)
Elixir Clistin, 4 mg. per 5 cc.

1. Johnson, H. J., Jr.: Am. Pract. & Digest. Treat. *5*:862 (Nov.) 1954.
2. Beale, H. D.; Rawling, F. F. A., and Figley, K. D.: J. Allergy *25*:521 (Nov.) 1954.

McNEIL
LABORATORIES, INC.
Philadelphia 32, Pa.

Advertisers Index

May, 1957

Abbott Laboratories . . . . . 11, 34–35, *Insert Facing Page* 40, 60–61, 74–75
Ames Company, Inc. 6, 22
The Armour Laboratories 66
Ayerst Laboratories 62, 77
Bilhuber-Knoll Corp. 73
Bristol Laboratories, Inc. 72–73
Burroughs Wellcome & Co., Inc. 33
Ciba Pharmaceutical Products, Inc. . . . . *Insert Facing Page* 16, 32, *Fourth Cover*
Desitin Chemical Co. 56
Eaton Laboratories 41, 67
Endo Laboratories, Inc. 80
Geigy Company 83
Hoffmann-La Roche Inc. . . . . 16, *Insert Facing Page* 24, 31, 71, 78, 82
Irwin, Neisler & Company 19, 36
Lakeside Laboratories, Inc. 27
Lederle Laboratories 8–9, 15, 37, 72
Eli Lilly and Company 64
Maltbie Laboratories Division, Wallace & Tierman, Inc. 18
The S. E. Massengill Company 30
McNeil Laboratories, Inc. 43–44, 84
Merck Sharp & Dohme 14, 52–53
The National Drug Co. 50
Organon Inc. 26
Parke, Davis & Company 21, 25, 29, 38–39, 49, 69
Pfizer Laboratories, Division of Chas. Pfizer & Co., Inc. 10, 45, 54, 70
Pharmacia Laboratories, Inc. 58
Riker Laboratories Inc. . . . . 42, *Third Cover*
A. H. Robins Co., Inc. 55
J. B. Roerig Co. 20, 40
William H. Rorer, Inc. 81
Sanborn Company 17
Schering Corporation 59
G. D. Searle & Co. 65
Sherman Laboratories 24
Spirt & Co., Inc. 76
E. R. Squibb & Sons, Division of Mathieson Chemical Corp. 4, 28, 46–47, 51, 63
The Upjohn Co. 48
U. S. Vitamin Corporation 68
Wallace Laboratories 23, 86
Wander Company, Division of Smith-Dorsey 79
Warner-Chilcott Laboratories 1
Winthrop Laboratories 2
Wyeth Laboratories 12–13

reports of clinical studies

1 "I have used meprobamate in my general psychiatric practice since April, 1955, and believe it to be [a] drug of choice for relief of tension, anxiety and insomnia."

Lemere, F.: Northwest Med. 54: *1098, 1955.*

2 ". . . the patient [taking Miltown] never describes himself as feeling detached or 'insulated' by the drug. He remains . . . in control of his faculties, both mental and physical, and his responsiveness to other persons is characteristically improved."

Sokoloff, O. J.: A.M.A. Arch. Dermat. 74: *393, 1956.*

3 "Of special importance is the fact that Miltown does not appear to affect autonomic balance—which in alcoholics is often unstable . . ."

Thimann, J. and Gaulhier, J.W.: Quart. J Stud. Alcohol. 17: *13, 1956.*

4 "The [relative] absence of toxicity, both subjectively and objectively, is an important feature in favor of Miltown. In addition, there were no withdrawal phenomena noted on cessation of therapy, whether it was withdrawn rapidly or slowly."

Borrus, J.C.: J.A.M.A. 157: *1596, 1955.*

5 "Miltown is of most value in the so-called anxiety neurosis syndrome, especially when the primary symptom is tension . . . Miltown is an effective dormifacient and appears to have . . . advantages over the conventional sedatives except in psychotic patients. It relaxes the patient for natural sleep rather than forcing sleep."

Selling, L.S.: J.A.M.A. 157: *1594, 1955.*

THE MILTOWN MOLECULE

Miltown®

THE ORIGINAL MEPROBAMATE

discovered and introduced

by Wallace Laboratories, *New Brunswick, N. J.*

2-methyl-2-n-propyl-1,3-propanediol dicarbamate—U. S. Patent 2,724,720

SUPPLIED: *400 mg. scored tablets. Usual dose: 1 or 2 tablets t.i.d.*

Literature and Samples Available on Request

Rauwiloid®

A Better Antihypertensive

"We prefer to use alseroxylon (Rauwiloid)

since it is less likely to produce excessive fatigue and weakness than does reserpine."[1] Up to 80% of patients with mild labile hypertension and many with more severe forms are controlled with Rauwiloid alone.

1. Moyer, J.H.: J. Louisiana M. Soc. *108*:231 (July) 1956.

A Better Tranquilizer, too

"...relief from anxiety resulted in generally increased intellectual and psychomotor efficiency with a few exceptions."[2] Rauwiloid is outstanding for its *nonsoporific* sedative action in a long list of unrelated diseases not necessarily associated with hypertension but burdened by psychic overlay.

2. Wright, W.T., Jr., et al.: J. Kansas M. Soc. *57*:410 (July) 1956.

Dosage: Merely two 2 mg. tablets at bedtime. After full effect one tablet suffices.

Best first step when more potent drugs are needed

Rauwiloid is recognized as basal medication in all grades and types of hypertension. In combination with more potent agents it proves synergistic or potentiating, making smaller dosage effective and freer from side actions.

Rauwiloid® + Veriloid®

In moderate to severe hypertension this single-tablet combination permits long-term therapy with dependably stable response. Each tablet contains 1 mg. Rauwiloid and 3 mg. Veriloid. Initial dose, 1 tablet t.i.d., p.c.

Rauwiloid® + Hexamethonium

In severe, otherwise intractable hypertension this single-tablet combination provides smoother, less erratic response to hexamethonium. Each tablet contains 1 mg. Rauwiloid and 250 mg. hexamethonium chloride dihydrate. Initial dose, ½ tablet q.i.d.

Riker LOS ANGELES

when blood pressure must come down

increased antihypertensive benefits

Lowering of diastolic and systolic pressures
Beneficial bradycardia
Increase in renal blood flow

low dosage of Apresoline

Combined with Serpasil, Apresoline is effective at an average daily dose of only 200 mg. Thus, side effects such as headache and tachycardia seldom occur.

versatility

Easier management of hypertension is made possible by two tablet strengths of Serpasil-Apresoline.

All patients to be given Serpasil-Apresoline may benefit from priming with Serpasil.

SERPASIL-APRESOLINE ***Tablets #2*** (standard-strength, scored), each containing 0.2 mg. of Serpasil and 50 mg. of Apresoline hydrochloride; ***Tablets #1*** (half-strength, scored), each containing 0.1 mg. of Serpasil and 25 mg. of Apresoline hydrochloride.

Serpasil®-Apresoline®

hydrochloride

(reserpine and hydralazine hydrochloride CIBA)

COMBINATION TABLETS

CIBA
SUMMIT, N. J.